POLYDORID POLYCHAETA AS PESTS IN CULTIVATED OYSTERS

A case study of polydorid infestation on a South African oyster farm, including a literature review and proposed Best Management Practices for the reduction of polychaete infestation in the culture of molluscs

Assignment presented in partial fulfilment of the requirements for the
degree Master of Philosophy (Livestock Industry Management:
Aquaculture) at the University of Stellenbosch

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DECLARATION

I, the undersigned, hereby declare that the work contained in this assignment is my own original work and that I have not previously in its entirety or in part submitted it at any university for a degree.

Signature: [redacted]

Date: **10 March 2006**

OPSOMMING

Die aanmelding van 'n wurm-agtige parasitiese infeksie van die Stille Oseaan oester, *Crassostrea gigas* met gepaardgaande stygings in mortalitiete op 'n oesterplaas op die Weskus van Suid Afrika, het aanleiding gegee tot 'n studie om die identitiet van die organisme, die strekking van die infestasie en die moontlike impak van die infestasie op die kondisie van die oesters te bepaal. Dit is vermoed dat die organisme 'n lid is van die spionid Polychaeta. 'n Literatuur studie van die groep is onderneem en die versamelde informasie word in die vorm van 'n oorsig aangebied. Die oorsig is so gestruktureer dat dit gebruik kan word as 'n praktiese handleiding vir oesterboere wat met die pes moet werk. Die oorsig behandel die sistematiek van die groep, die lewenspatrone, die impak van die organsime op oesters en die beheer van die organisme.

Die organisme is geidentifiseer as 'n lid van die spionid polychaeta, en meer spesifiek 'n lid van die genus *Polydora,*'n bekende pes van oesters. Daar is gevind dat die organisme teen verskillende konsentrasies voorkom op die plaas en dat die konsentrasies korrelleer met die ouderdom van die oesters. Die hoogste konsentrasies is gevind in die oudste oesters op die plaas en die oesters is ook heel moontlik die oorsaak van die infestasie aangesien hulle van 'n ander oesterplaas oorgeplaas is. Die kondisie van al die oesters was goed in vergelyking met internasionale standaarde (kondisie indeks > 10). ANOVA analiese van die data het geen beduidende veskil gevind tussen die kondisie van besmette en onbesmette oesters nie. Dit kan dus nie onteenseglik bewys word dat die mortaliteite toesgeskryf kan word aan die *Polydora* infestasie nie. Alhoewel geen enkele rede gevind kan word vir die mortaliteite nie is dit heel moonlik toeskryfbaar aan 'n kombinasie van intrinsieke en eksterne faktore.

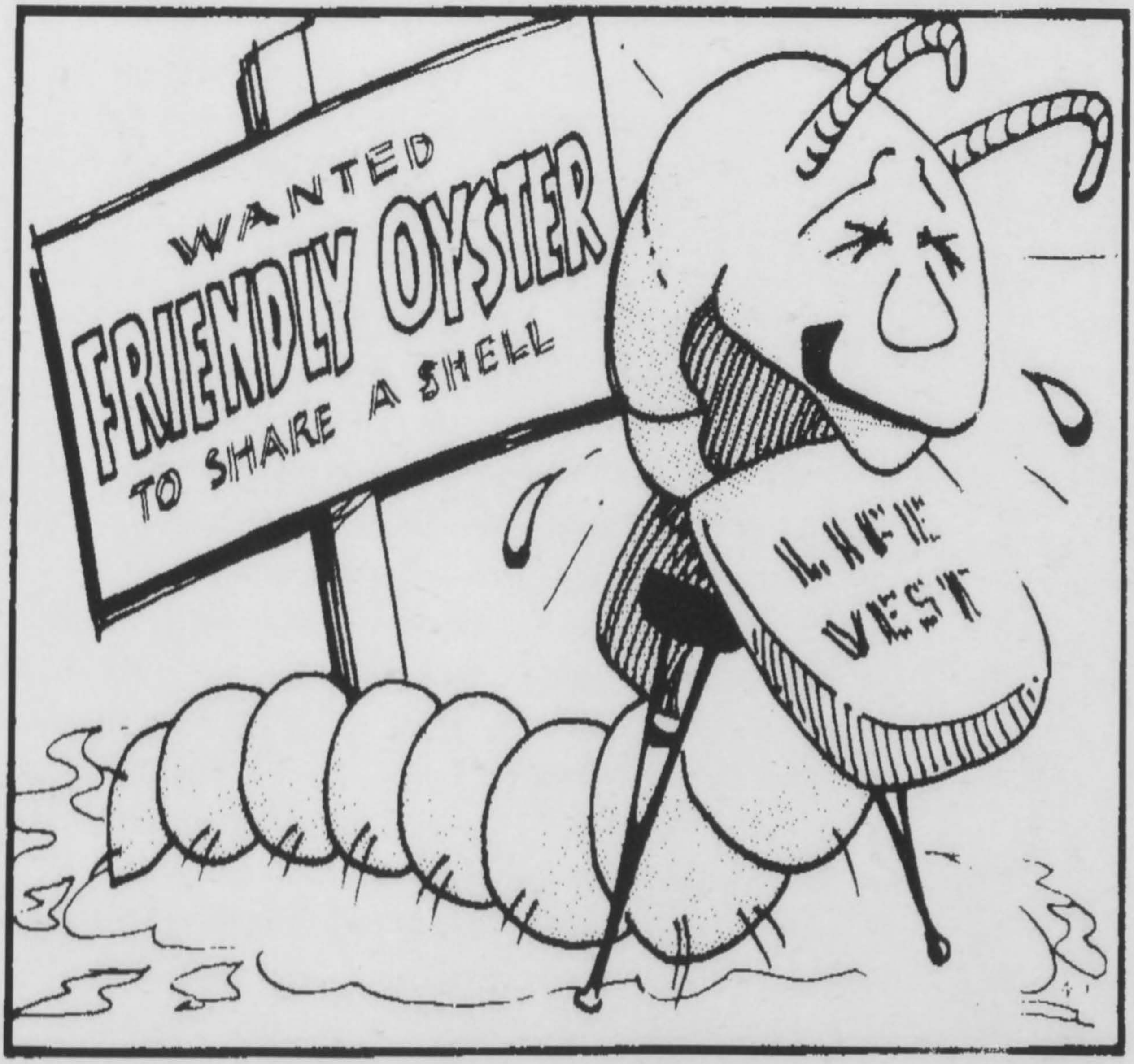
Die implementering van kwaliteitsbeheer sisteme soos HACCP op akwakultuur plase is van kardinale belang vir sukses. 'n Stel Beste Bestuurs Praktyke (BMP) vir die groei van oesters word voorgestel en spesifieke verwysing word gemaak na die kritiese beheer punte soos vereis deur HACCP. Die huidige bestuursisteme van die plaas word ook ondersoek en dit is bepaal dat die totale gebrek aan effektiewe bestuur die hoof rede is vir probleme wat die plaas ondervind.

SUMMARY

Reports of a wormlike “parasitic” infestation of pacific oysters, *Crassostrea gigas* combined with an increased mortality on a land based oyster farm on the west coast of South Africa prompted an investigation as to the identity of the infesting organisms, the extent of the infestation and the impact on the condition of the oysters. As the infesting organism was suspected to be a member of the polydorid complex of the spionid polychaeta, a literature study was conducted and the information gathered, compiled in the form of a review which also serves the purpose of a practical guide for aquaculturists who have to deal with polydorid infestations. The review summarizes information on the systematics, life history, impact and control of the group.

The infesting organism was identified as a member of the spionid polychaeta and specifically the genus *Polydora,* a known pest in bivalve molluscs. It was found that the organism showed varied levels of infestation in the spatially and age segregated oyster population on the farm with the oldest animals the heaviest infected and also the possible source of the infestation as they were brought onto the farm from another oyster farm. The condition of all the animals in the different cohorts on the farm was good compared to international standards (condition indexes (CI) > 10) ANOVA analysis of data could find no significant difference between the condition of infested and uninfested oysters and therefore it cannot be conclusively proved that the increased mortalities can be ascribed to the polydorid infestation. Although no single cause seemed to be implicated the mortalities can probably ascribed to a combination of several extrinsic and intrinsic factors.

The implementation of quality control systems such as HACCP on aquaculture farms is critical for successful cultivation. A set of Best Management Practices (bmp) for the culture of bivalve molluscs is proposed, with identification and special reference to the critical control points as required by HACCP. The current practices on the farm in question were also investigated and it was concluded that the absolute lack of management on the farm was the primary cause for the problems experienced.



Courtesy Margaret Skeel (1977)

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Finally, I wish to dedicate this thesis to my father, a great man, father and scientist, who are sadly not here to see the final result. You were always my inspiration and your unwavering support and belief in me carried me through times good and not so good. We all miss you and you will always be in our hearts.

Chapter 1 A REVIEW OF THE POLYDORID COMPLEX OF THE SPIONID POLYCHAETA.

1.1 Introduction

Two families of the phylum Polychaeta, the Spionidae (mud-worms) and the Sabelidae (fan-worms) have members who are found to live commensally with economically important molluscan mariculture species. The focus of this study will be the polydorid complex of the Spionidae that comprises the related genera *Polydora, Boccardia,* and *Pseudopolydora*, with the first two genera both found in Southern African waters (Day, 1967). Polydorids are widely spread, having been reported from both the Northern and Southern Hemispheres. The polydorids are common but generally inconspicuous small marine polychaetes that bore into hard calcareous substrates or constructs sand grain tubes in soft sediments. They are best known for this shell boring ability and are the only spionids capable of this. The mechanism of this boring is the subject of considerable speculation. This boring activity, combined with the accumulation of silt, which leads to the formation of mud blisters through the reaction of the mollusc to the worm forms the main focus of this study.

Members of the Sabelidae are also important aquaculture pests having impacted heavily on especially cultured abalone both in South Africa and other regions. This group has been discussed in detail by Simon (Simon *et. al.*, 2004), Ruck (Ruck and Cook, 1998), Lleonardt (Lleonardt *et. al.*, 2003) and many other authors and will not be dealt with in this study. The treatment regimes and culture system management of the polydorids as presented in this thesis can also be applied to control sabellids.

The polydorids have been the cause of massive losses of natural and cultivated populations of molluscs with the earliest reports dating back to Whitelegge (1890) and Roughley (1922). These infestations are not limited to oysters and also impact on other economically important mariculture shellfish species. It has been reported in mussels (Lebour, 1907), scallops (Bower *et. al.*, 1992), abalone (Lleonardt *et. al.*, 2003) and clams (Tinoco-Orta and Caceres-Martinez, 2003). The systematics of the polydorids is still not clearly defined and there are significant variation in the ecology, reproductive biology and opinions as to the impact of this group on mariculture.

The next section presents a summary of the current knowledge of members of the polydorid complex that impact on mariculture shellfish species and an overview of current methods of control of these organisms in production systems.

1.2 Systematics of the Polydorid complex

Extensive literature exists on the systematics of the polydorid spionids from both Hemispheres (Hartman 1959, 1965; Okuda 1937; Foster, 1971; Read, 1975; Radashevsky, 1993; Pillai, 1965; Sato-Okoshi and Okoshi, 1997). As with the systematics of most invertebrate groups various authors have different systematic classifications for the group. Read (1975) describes the generic arrangement of the polydorid group as not yet stable or completed satisfactorily which is a situation which also occurs in some other groups within the family Spionidae. He states that the generic breakdown is based on some key characters including the shape of the prostomium and peristomium, the position number and type of the gills or branchiae, the degree of attachment of notopodial lamellae to the branchiae and the position and type of hooded hooked setae. Foster (1971) suggests that the position of the branchiae is the most important generic differentiation character because the presence or absence and type of hooded hooked setae in the notopodia are generic characteristics in other spionids.

Read (1975) identifies three genera, *Polydora, Boccardia* and *Pseudopolydora* within the polydorid complex. In a later revision, Radashevshy (1993) identifies four genera namely *Boccardiella* (*Boccardia syn.*), *Neoboccardia, Polydora* and *Pseudopolydora.* A plethora of species from around the world has been identified but many seem to be re-descriptions of earlier described species.

Members of the group exhibit various lifestyles including inhabiting mud or sand tubes on soft bottoms, fouling of different hard substrata and boring into various calcareous structures. Boring and non-boring or tube dwelling species are generally recognized on the basis of their boring activity. However no structural adaptation distinguishes the boring from the non-boring forms (Radashevshy and Hsieh, 2000) and thus the lifestyle of the species cannot be ascertained from it's morphology.

From a systematic's point of view it is required in practice that the aquaculturist should be able to identify the infesting polychaete by first differentiating between sabellids and polydorids and then to at least the genus level for the polydorids. Although the systematic identification is based mostly on fine anatomical differences, identification should be fairly easy for the aquaculturist using a ordinary dissecting microscope. Identification is best made working with live specimens as preservation may mask some of the features by rendering some structures opaque. Methods of extracting live specimens are described in chapter 2 section 2.2.5.

Differentiation between sabellids and polydorids is based on the structure of the prostomium and head of the animal with the polydorids having only two palps or tentacles while the sabellids have a indistinct prostomium and a highly modified head with either frilly membranes, setae, buccal tentacles or a branchial crown (Figure 1).

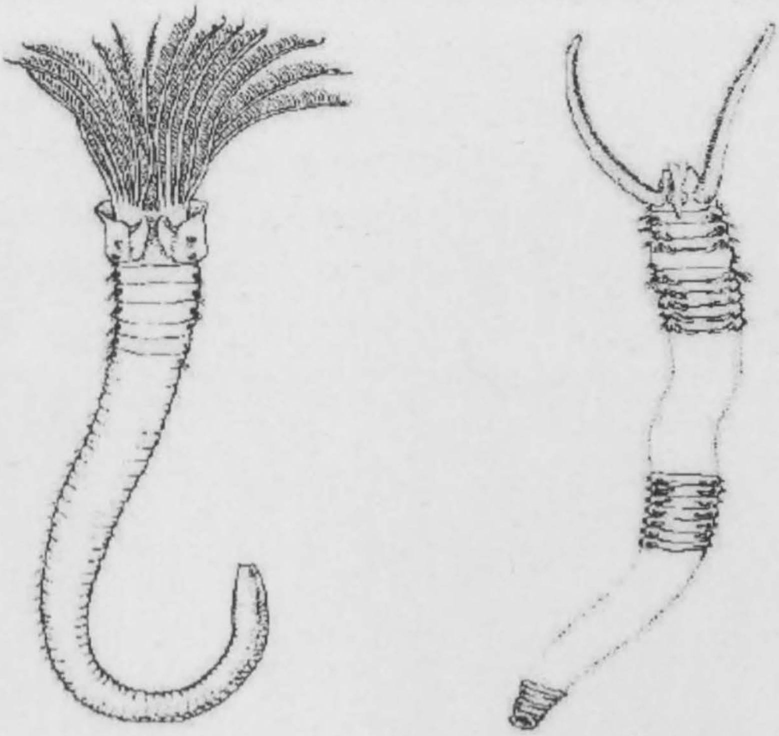

Figure 1 Anatomical differences between sabellids (left) and polydorids (right)

For further identification of the polydorid complex certain key segments of the body (setigers) are used. All the generic groups have a curved row of heavy spines of two types or one type with companion setae (bristle hairs) on the fifth setiger and the modification of the fifth setiger and the placement of the branchiae (gills) are used for classification (Figure 2).

The genus *Polydora* is identified by the anteriorly rounded, incised or bilobed prostomium, the enlarged fifth setiger with a curved row of heavy spines of two types or one type with companion setae. Branchiae is absent on the first five setigers with the first visible from setiger 6 and continuing a variable number of segments posteriorly.

The genus *Boccardia* is identified by the anteriorly rounded, incised or bilobed prostomium, the enlarged fifth setiger with a curved row of heavy spines of two types or one type with companion setae. The branchiae or gills begin on the second setiger (segment) is absent on setiger number five and continues a variable number of segments posteriorly.

The genus *Pseudopolydora* is identified by the anteriorly rounded, incised or bilobed prostomium, the absence of a noticeably enlarged fifth setiger although the curved row of heavy spines of two types or one type with companion setae (bristle hairs) is still present. Branchiae is absent on the first five setigers with the first visible from setiger six and continuing a variable number of segments posteriorly.

Identification to species level becomes more difficult with small variations in the arrangement, absence or presence and structure of the neuropodial hooded hooked setae and notosetae spines and bristles determining species. The ability to identification the organisms onto the species level is of lesser importance to the aquaculturist as it add no further value in relation to the control of the animal. A simplified key for the identification of members of the group is provided in Table 1.

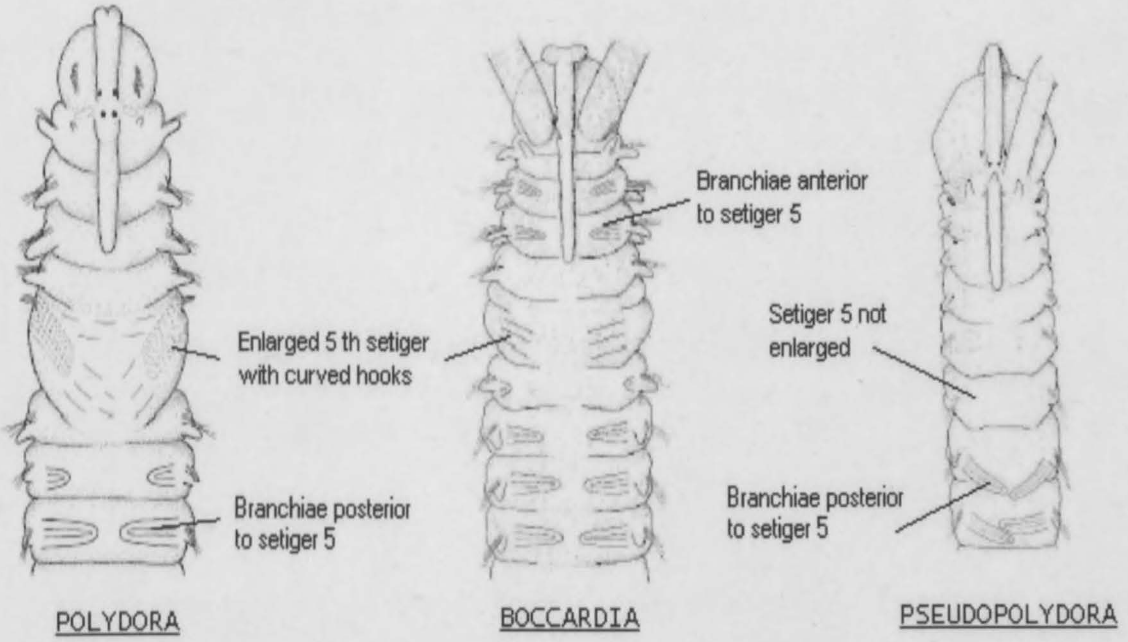

Figure 2 Anatomical characteristics of the three genera: *Polydora, Boccardia* and *Pseudopolydora*

Day (1967) identified three species of *Boccardia* (*B. legerica, B. polybranchia* and *B. pseudonatrix*) and 12 species of *Polydora* (*P. capensis, P armata, P.hoplura hoplura, P. hoplura inhaca, P. flava, P. caeca, P. ciliata, P. normalis, P. giardi, P. maculata, P antennata* and *P. kempi*) in South African waters and he mentions that most of the shells of abalone on the Cape coast are riddled with *Polydora*.

Table 1 A simplified key for the identification of the Spionidae and the Sabelidae

1.	1.1	An anterior region of nine or more flattened uniramous segments followed posterior regions of biramous segments.	**Chaetopterus spp.**
	1.2	First 9 Segments not uniramous	To 2
2.	2.1	Prostomium and peristomium well developed	To 3
	2.2	Prostomium indistinct and head highly modified with either frilly membranes, setae, buccal tentacles or a branchial crown	**Sabelidae**
3.	3.1	Peristomium with one or more pairs of long grooved palps. Branchiae usually starts on the first or second setiger	To 4
	3.2	Head without appendages, gills if present do not start before the 5 th setiger	**Orbinidae**
4.	4.1	Parapodia well developed. Never more than one pair of grooved palps. (Spionidae)	To 5
	4.2	Parapodia reduced to mere ridges. Body cylindrical. Gills long and filiform	**Cirratulidae**
5	5.1	5th setiger clearly enlarged with curved row of heavy spines of two types or one type with companion setae	To 6
	5.2	5th setiger not clearly enlarged. Curved row of heavy spines of two types or one type with companion setae	**Pseudopolydora spp.**
6	6.1	Branchiae (gills) begin on the second setiger (segment), are absent on 5th setiger and continue posteriorly.	**Boccardia spp.**
	6.2	Branchiae (gills) absent on first 5 setigers, starts on setiger 6 and continues posteriorly	**Polydora spp.**

Adapted from Day (1969)

1.3 The Life History of the polydorid complex

The life history patterns of the polydorid complex have been described as opportunistic or r – selected (Grassle and Grassle, 1974) and their reproductive pattern as semi-continuous or continuous breeders (Olive and Clark, 1978). The group shows considerable variation in gamete morphology, modes of sperm transfer and types of larval development (Gudmundsson, 1985). However parameters which characterize opportunistic species, such as low reproductive age, high fecundity and short lifespan apply to the polydorids in a varying degree and are too complex to generalize for the group as a whole. The large degree of variation that is observed in the life history patterns between species and within populations of the same species in various locations, is perhaps due to adaptation to variable local living conditions.

1.3.1 Habitat

Polydorids are known to live in a very wide variety of habitats, both polluted and unpolluted. They are incredibly cosmopolitan and widely distributed with single species ranging from Europe to China (*P. agassizi*), from the mudflats of the east, west and gulf coast of the USA to Argentina, Europe and Australia. (*P. cornuta*), Brazil, South Africa, the Subantarctic, to Europe and Australia (*B. polybranchia*) and from South Africa to the Mediterranean, Ireland and Alaska (*P. ciliata*). Habitats can be very varied in certain species while very specialized in others. Members of the group can be very loosely be classified as one of the following, free-living tube dwellers, borers or commensals. Although this classification is problematic, with some authors accepting more than one lifestyle in one species (Blake, 1969, 1971) while others believe in species-specific habitat preference (Sato-Okoshi and Okoshi, 1997), it does provide some insight into the different habitats of the polydorid complex.

Free-living tube dwellers can be found in mud or sand tubes on soft bottoms or fouling different hard substrata. These substrata can range from various types of rocks, harbour structures and other man-made surfaces. These tube dwellers can form dense populations that may reach several thousand per square meter. Animals are found in estuaries and the open sea and range from intertidal zones down to depth in excess of 25 meters.

Commensals do not bore but form their tubes on the surfaces of other species like *P. agassizi* that have been found on the ventral anterior carapace and legs of horseshoe crabs and *P.cornuta* inside the tubes of the onuphid *Diopatra sugokia* (Radashevshy and Hsieh, 2000). Some species have been reported to foul bivalve and gastropod shells without boring while others are found on hermit crab shells and commensally on sponges.

Most boring polydorids are associated with various gastropod and bivalve molluscs. *P. ciliata, P. websteri* and *P. hoplura* are the species most often recorded in the literature to be infesting bivalve shells (Blake and Evans, 1973). This is most probably because they seem to be the species that impact most on commercially valuable molluscs and are therefore most frequently encountered and studied. These calcareous borers are however not limited to living molluscs but also forms burrows in empty shells and other calcareous substrates like the calcareous tubes of serpulids, crusts of coralline algae and in the shells of barnacles. Some calcareous borers also bore into sponges (*P. caruncutata*) but are morphologically identical despite habitat differences (Radashevsky, 1993). *P. spongicola* is also a borer of sponges and have been found in a variety of sponges in various parts of the world. About 11 species have been reported to bore into corals (Martin and Britayev, 1998). Two of the coral boring species have also been reported to bore into molluscan shells.

1.3.2 Feeding

Spionid polychaetes feed at the sediment–water interface with a single grooved pair of ciliated palps that arise from the peristomium. They have been classified as selective surface deposit feeders (Santos and Simon, 1974), suspension feeders (Dorsett, 1961) or as both deposit and suspension feeders (Fauchald And Jumars, 1979).The worms extend the palps from the opening of the burrows and feed in two different ways. If the palps come into direct contact with the sediment, particles are picked up in the food groove and transported by ciliary action to the everted pharynx (Dauer, Maybury and Ewing, 1981). The palps can also be extended and held erect in the water column (personal observation), catching suspended particles it comes into contact with. Spionids have been reported to feed on sediment particles, planktonic organisms and meiobenthic organisms (Daro and Polk, 1973). Waste is expelled either as unconsolidated faecal material which is swept out of the U–shaped tube by the respiratory current or as faecal pellets. Spionid polychaetes are not parasitic and do not feed on its molluscan host.

1.3.3 Reproduction

Many polydorids are reproductively active throughout most of the year and can therefore be classified as semi-continuous breeders. Sexes are generally separate but hermaphrodites have been found on occasion (Zajac, 1991). The males and females produce gametes in segments within the middle half of the body (gametogenic setigers). Males release spermatophores onto the sediment surface. Females then use their palps to direct the spermatophores into their tubes for storage in seminal receptacles. Fertilization is thought to occur as eggs are deposited in capsules. Hannerz (1956) recognized three main developmental types within the Spionidae: (I) Development entirely pelagic, (II) development entirely or partially in structures produced by the female, (III) development in the female body. The polydorid complex belongs to the second category.

Females lay eggs in a string of capsules they attach to the inside of the maternal tube. Each capsule corresponds to a reproductive segment of the female (Blake 1969). Once the set of capsules have been deposited a new batch of eggs can begin do develop in the coelom. Three types of egg capsules are found among polydorids. The simplest is a hollow cylinder attached to the parental tube by thin extensions along its length (Fig 3A). The second type is found in most species and consists of small capsules joined beadlike in a string (Fig 3B). Each individual capsule is attached to the wall of the adult tube by either one or two stalks. The third type is found *Boccardia* spp. and here the capsules occur singly and are not connected to each other and are attached to the wall of the tube by two thin extensions (Blake, 1969).

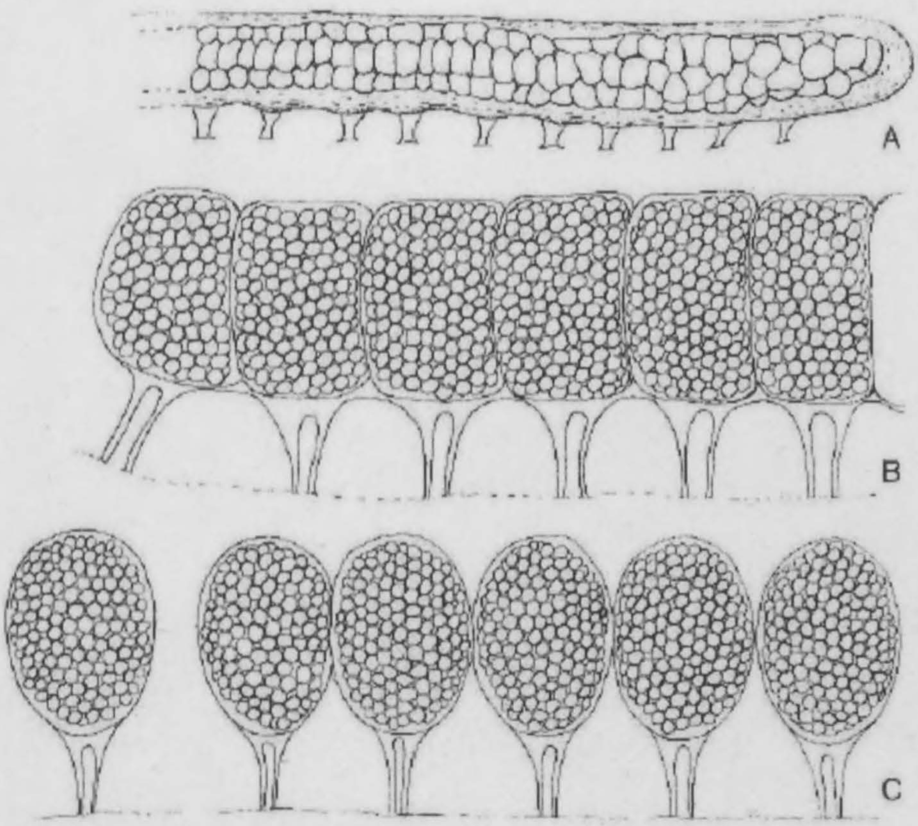

Figure 3 Different types of egg capsules of Polydora (from Blake 1969)

Larval development in the egg capsules varies between species. Two types can be distinguished. Adelphophagia or nurse egg feeding (Type I) occur in several species (Blake 1969). The presence of nurse eggs (unfertilised eggs in the egg capsule) provides the developing larvae with an extrinsic food source within the capsule. Larvae that are provided for in this way stay in the egg capsules longer and the pelagic phase in either short or omitted entirely. Larvae appear from the egg capsules at the setiger 10 to 15 stage. Larvae of this type are approximately 800 - 1000 μ in length. Species that have no nurse eggs (Type II) become pelagic when they reach setiger stage three to six and have utilized their intrinsic yolk supply. These larvae are much smaller and measures approximately 350 μ.

The ability for a single species to vary its mode of reproduction within its geographical range has been termed "poecilogony" by Giard (1905). This phenomenon has been observed within several species of the polydorid complex (*P. qaudrilobata, B. proboscidae and B. columbiana)* with Type I larval development in certain populations and Type II in others.

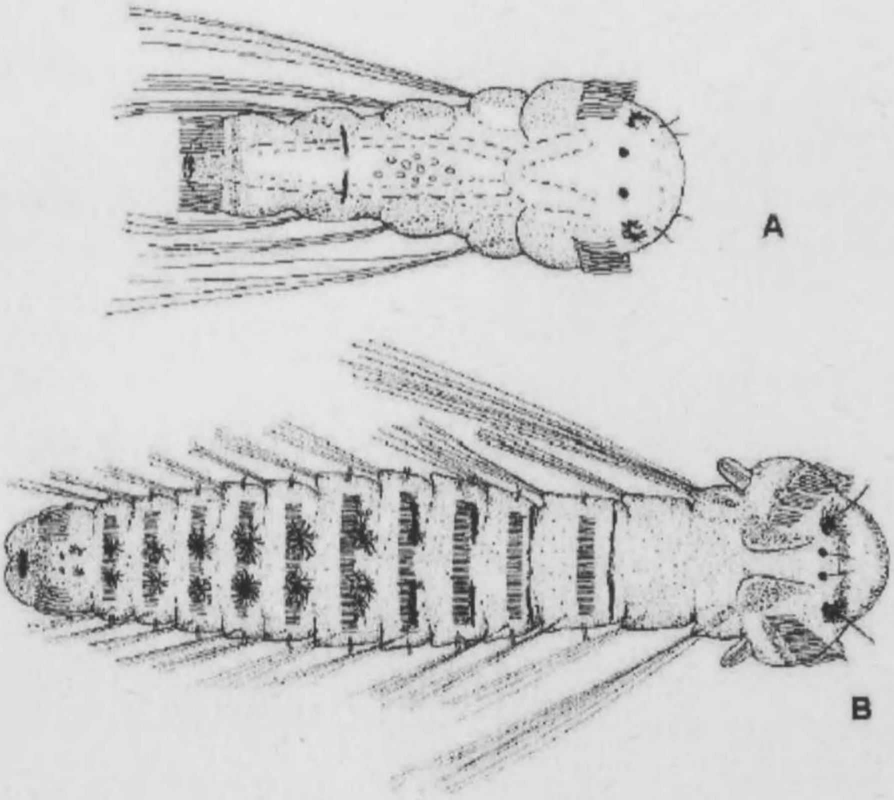

Figure 4 Larval stages of *Polydora websteri*: A. Setiger 3 and B. Setiger 12 (from Blake 1969).

Planktonic larvae are photopositive during the early stages (they have eye spots) and become photonegative just before settlement. Metamorphosis occurs between setiger stage 15 and 20. The presence of fine silt especially from a parental source or shell substrates easily induces the larvae to metamorphose and settle. Larvae, which are ready to settle, measure approximately 1500 –2000 μ.

First reproduction can take place within 35 days of settlement. Smallest individuals with sexual products in coelom have between 35 and 50 segments (Gudmundsson, 1985). Females can produce consecutive broods. In most species preparation for egg laying starts in autumn and egg laying (capsule deposits) commences in early spring, but has been observed throughout the year, except for winter. Larval development is quick (3-4 weeks) with increase in temperature accelerating growth. By mid summer larvae are found to be present in plankton. Recruitment slows down in late summer and ceases during winter. Egg capsules can contain from 35 to in excess of 150 eggs depending on species and larval development type. Up to 2200 eggs per batch have been recorded (Gudmundsson, 1985, Zajac 1991, Blake 1969). Polydorids reach maturity within a few months and their lifespan is longer than a year and are therefore potentially polytelic.

1.3.4 Settlement, mud blister formation and growth

Settled larvae appear in the benthic population when they consist of about 20 setigerous segments. They are found throughout the year with highest numbers from late spring to early summer (Gudmundsson, 1991). On settlement the larvae immediately begins to construct a burrow.

1.3.4.1 Burrow Types

Three main types of polydorid burrows have been described. Type I: Surface fouling occurs when the worm settles on the surface but do not penetrate. The worms accumulate a thick layer of mud around themselves and over the substrate. The individual worms extend their burrows beyond this mat as two neat, round, mud coloured tubes. Type II: U–shaped burrows penetrate the structure of the shell and consists of a elongated U with the arms parallel and quite close together. Type III: Mud blisters are masses of mud accumulated by just settled worms on the inside of the host shell. The host reacts by first secreting over the mud a roof of proteinaceous conchyolin and later a layer of nacreous material. The worm occupies the mud filled chamber and communicates with the exterior via a pair of tubes at or close to the periphery of the shell (Blake 1973).

1.3.4.2 Method of entry and mechanisms of burrow formation

There appears to be considerable disagreement in the literature concerning both the method of entry into the shell and the tube building behaviour of this group. The information available from the literature concerning the route of invasion followed by the mud blister formation is contradictory but the most obvious explanation is that the Polydorids are capable of penetrating the host shell by using either of the modes of invasion that has been described. Contradictory observations by different authors working with same species may well be explained in this way.

External borers

Some authors describe boring through the shell structure from the external surface (settling larva have been observed on the surface of shells (Blake, 1973)) using metabolic acids and mechanical abrasion. Lankaster (1868) was the first person to treat the actual boring of polydorids and proposed that the burrowing was accomplished by chemical means with the worm secreting acid derived from segmental glands later termed "*pouches glanduleuses*" by Claparede (1870). McIntosh (1868) differed of opinion because the worms were also found in non-calcareous substrates and he favoured a mechanical mechanism. The difference of opinion as to the mechanism of penetration continued well into the 20th century with Sonderstrom (1920) emphasizing mechanical abrasion by the heavy spines found on the fifth setiger and postulates that it was a joint effect between the secretion of an acid and mechanical abrasion of the spines. In a later paper he however changed his mind and sided with a pure chemical action. Hannerz (1956) found that *P. ciliata* larvae possessed a pair of opaque grey glands, ventral to the fifth setiger spines and he postulated that these glands secreted a substance that facilitates the initial boring by the worm. He also noted the strong musculature of the fifth setiger and favoured a chemical and mechanical approach. Due to the fact that the glands were exclusively larval he contended that boring in adults was accomplished with only the spines.

Hempel (1957) determined that free-living specimens bored into clay using the fifth setiger spines. She also noted that the spines showed significant wear in shell boring specimens and favoured a purely mechanical mechanism. Dorsett (1961) once again favoured both mechanical and chemical means. No specific acid was identified but the use of a sequestering or chelating agent linked with the biochemistry of mucous was suggested. Haigler (1969) conducted an elegant range of experiments in which she showed that worms that have had their spines removed could still bore. This discovery coupled with the observation made by Evans (1969) that *P. conchrum,* which constructs long branching burrows, enlarged all branches of the burrow at the same time and by Zottoli and Carriker (1974) who after prolonged observation of *P. websteri* saw no use of the spines to either mechanically remove substrate or to enlarge burrows finally put the mechanical theory to rest.

The structure of the burrow system shows variation but the construction of the burrow follows a similar pattern. The larvae settle on the surface of the shell. The newly metamorphosed juvenile excavates a shallow depression, constructs a silt tube and with growth deepens the depression. With continued growth and boring, the burrow is deepened considerably and is formed into different patterns depending on species. *P. commensalis* forms a shallow depression, which is roofed over with a layer of calcite. *P. cilliata* forms a U-shaped depression while *P. websteri* forms a pear or sack shaped burrow. *P. concharum* and *P socialis* forms long twisting burrows, which is nearly impossible to trace when fully developed. As the burrow deepens it may penetrate into the mantle cavity where the mollusc will then deposit a nacreous layer over the worm to protect itself.

Internal settlers

Other authors (Handley and Bergquist, 1997; Skeel, 1977; Whitlegge, 1890) postulated that the worm did not burrow at all and that the settling larvae either swims into the mollusc or is sucked in by the inhalant feeding currents. The larvae move to the mantle cavity or creeps between the mantle and shell where it attaches and begins construction on a burrow by accumulating sediment and shaping this burrow with secreted mucus.

The burrow is constructed to open on the margin of the shell where the worm feeds. This accumulation of mud irritates the host who then deposits first a thin layer of a conchyolin and then later a nacreous layer and thereby forming the mud blister. Handley and Bergquist (1997) observed three types of mud blisters within a single oyster (Figure 5). Blisters exhibiting a thin conchyolin or calcareous covering were termed “new mud blisters”, of which two forms were distinguished. The most common ‘larval new mud blister’ contained juvenile *P.websteri* and was presumably formed during larval settlement. They typically consisted of a soft thin blister of greenish-brown conchyolin terminating at the periphery of the shell where the mud blisters opened with a single tube from which the worms emerged to feed and defecate. At muddy sites close to the substratum these tubes were extended into long chimneys of aggregated sediment. The second new mud blister type, ’internal new mud blister’, was not connected to the shell periphery and contained worms which had bored through the surface of existing mud blisters or from the external surface of the oyster. This once again supports the idea that the worms can use either method of burrow construction. In some cases Spionid burrows and worms could be seen within the translucent shell matrix. Mud blisters covered by a thick calcareous layer were termed 'old mud blisters'. Large oysters often contained multiple layers of blisters, the youngest formed closest to the shell opening. In heavily infested oysters containing multiple overlapping mud blisters, many spionids were often found inhabiting a single mud blister with a labyrinth of interconnecting burrows containing both *Boccardia* and *Polydora* species.

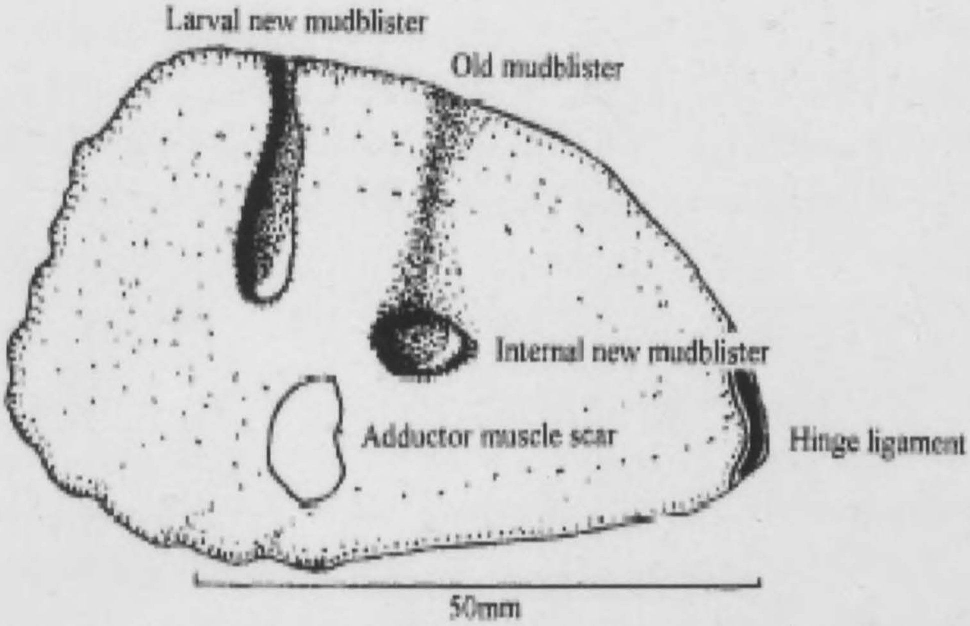

Figure 5 Shell mud blister types produced by *Polydora websteri* infestations inside the mantle cavity of Pacific oysters (from Handley & Bergquist, 1997).

In a study at Milford Harbour, Connecticut, Long Island, Loosanofff and Engle (1943) introduced various size classes of oysters from deep-water beds of Long Island Sound where Polydora is very uncommon (less than 2 %). Within 2 years all were heavily invested with *P. websteri* with the oldest class the heaviest infected, but no correlation between the degree of infestation and the age of oysters could be assumed as larger oysters have more available surface area.. Tubes were U-shaped with double holes on exterior surface of the oyster. In cross section the shells showed up to 6 layers of blisters superimposed on top another. With the worms ranging in size from biggest at bottom and youngest on top. Although conditions in the shell were overcrowded, the worms all appeared to be alive.

1.3.4.3 Relationship between environmental conditions and the degree of infestation

The literature identifies certain environmental / habitat conditions, which leads to higher levels of infestations. These conditions include variation in salinity, level of sedimentation and intertidal exposure.

Salinity

Loosanoff and Engle (1943) found that the bottom living oysters from the same area showed much less infestation than those suspended in trays with many showing no infestation at all. Suspended oysters were still covered with heavy layer of deposit consisting of silt, mud and living and dead plankton. The study area showed clear salinity stratification and the bottom had higher salinity than top. Stephen (1978) found a significant decrease in the level of infestation as the salinity decreased (to nearly freshwater) due to monsoon rains. It would appear that the polydorids are most comfortable in a salinity range of 25 – 30 ppt (Skeel 1977). Bishop and Hooper (2005) found that treatment of juvenile oysters with a hyper saline solution dramatically reduced infestation rates.

Sedimentation

Handley and Bergquist (1997) suggests that growing oysters at least 0.5 meters above the substratum will reduce the chances of infestation and although it is believed that mud worms need sediment they could not show a clear correlation between the level of sedimentation and the level of infestation. Regular cleaning of oysters also had no effect on level of infestation. Skeel (1977) considers silt load an important factor and areas with a heavy silt load problematic. Off the bottom culture can help polydorids escape predation and excessive siltation if they should settle on these oysters. Heavy silt loads can then provide the worm with the building material it needs.

Intertidal exposure

Caceres-Martinez *et. al.* (1998) found that intertidal exposure contributed significantly to the control of polydorid infestation with oysters being regularly exposed showing far lower levels of infestation. Wisely *et. al.* (1979) found a similar reduction in infestations when oysters cultivated in deep water were regularly exposed to air. Handley and Bergquist (1997) also found a positive correlation between decreasing intertidal exposure and increased prevalence of new larval mud blisters. Skeel (1977) suggests regular daily exposure to control infestation and also states that single longer-term exposure is less successful.

1.4 Impact on Cultivated Molluscs

In cultivated oysters mud blisters negatively affect the half shell market because the blisters can be punctured and releases anaerobic metabolites including hydrogen sulphide. It also renders the oyster shell brittle and easily broken during shucking, packaging and transport.

The effects that spionid polydorid infestations can have on oysters have been confusingly portrayed in the literature. Some studies have detected a parasitic effect resulting in decreases in condition due to infestations (Lunz, 1941) whereas others imply commensalism with no significant effect (Stephen, 1978) or even greater condition (Schleyer, 1991). Loss of condition may be expected in mud worm infested oysters as mud blisters within the mantle cavity reduce the internal volume and disturb the feeding current of the oysters (Korringa, 1951). Blistered oysters must increase shell deposition to regain shell volume and to smooth the internal surface around the mud blister. This would lead to an increased channelling of available energy resources away from growth to blister deposition and repair.

Handley and Bergquist (1997) however found that the condition index of infested oysters were negatively correlated to the level of infestation, with Handley (1998) criticizing the use of static condition indexes as inappropriate if confounded by the loss of shell volume and increased shell deposition resulting from infestation. He goes on to conclude that in terms of aquaculture production, the impacts of Spionid infestation on oyster meat production was negligible if one ignores the loss in value to the half-shell market due to the presence of blisters.

Caceres-Martinez, *et al.* (1998) found no correlation between the condition of the oysters and either blister number or blister area. They however suggest that the control of spionids is not only important because of possible negative effects on the CI but also for marketing. Boscolo and Giovanardi (2002) reported heavy infestation of the Manila clam (*Tapes philippinarum)* by *P. ciliata*, but reports that the clams were not in a "suffering state" with the flesh condition not poor and the animals actively producing gametes. The shells however were weak and bad from a marketing point of view.

Bower *et. al.* (1992) reported heavy infestations of *P. websterii* in Japanese scallops leading to mortalities of 84 %. Most surviving scallops were stunted and shell re-growth was abnormal. It is possible that mortalities in scallops are so high due to the weakening of the shell structure underneath the adductor muscles, which makes up a large percentage of the body mass of the scallop.

Loosanoff and Engel (1943) found that oysters despite heavy infestation were in very good condition and were unusually "fat" and large in size. With the condition index (CI) as high as 10.1 % as compared to 5.7 for the oldest and 13.7 as to 11 for the youngest. Stephen (1978) found that infestations did not impact on the condition of the oysters but this was not quantified. Kent (1979) found that heavy infestations reduced the condition of mussels but milder infestations had no significant effect. Wargo and Ford (1993) also found that the tissue condition and stores of energy reserves were negatively influenced by heavy infestations (45 % or more of shell surface).

Once again the literature is not clear but out of the available studies it can be concluded that polydorid infestations do impact on the condition of the oyster if the infestation is heavy and the animal have channelled significant resources into building protective structures against the invaders.

From an commercial aquaculture point of view the impact is far greater with unsightly blisters, the psychological idea of a worm in the oyster and concomitant pollution if the blister should be broken during opening causing buyers to reject the oysters or at the very least not paying full market price for the animals. (J. Booysen pers. comm.). The main impact of the infestations is therefore financial and any farmer should take all steps possible to minimize or prevent possible infestation.

1.5 Treatment of cultivated molluscs

The literature provides various methods that have been successful to a varying degree. None of the methods however were 100 % successful so prevention is far more important that cure. Treatment methods must be practical and not damaging to the environment. The use of chemicals to treat large numbers of organisms is therefore not sound.

Korringa (1952) claims to have had success in eliminating most off the Polydora by bathing the oyster for 16 hours in fresh water or for 3 hours in 0.5 $g.l^{-1}$ solution of the ammonium salt of dinitro-orthocresol in seawater. According to Korringa (1952) the oysters did not suffer noticeably from these treatments and soon showed better growth and whiter shells. No data on specific mortality or reduction in numbers of polydorids is presented.

Mackenzie *et. al.* (1970) tested a number of chemical compounds either as vermifuges (chemicals that causes worms to emerge from their tubes) or vermicides (chemicals that kill worms in their tubes but not cause them emerge). Over 30 compounds caused worms to emerge but most of these compounds (many are chlorinated solvents) are today considered toxic and classified as marine pollutants. O-dichlorobenzene was considered the most effective as it causes high emergence at low concentrations (100 mg.l^{-1}). The authors do however consider salt the most practical chemical and they found mortalities of polydorids of between 87 and 98 % after a 10 – 15 minute dip in a saturated salt solution followed by 15 minutes or more of drying.

Skeel (1977) tested three chemicals, magnesium chloride, phenol and MS 222 and found that all three caused enough irritation to drive the worms from their burrows without affecting the oyster that reacts to the chemicals by closing tightly. Due to the potential environmental impact of chemical treatment she however suggests that physical and biological control methods should rather be investigated. Current literature however, makes no references to these methods of control.

In a South African study, (Nel et. al, 1996) focused on the manipulation of salinity and temperature as methods of control. They exposed the oysters to fresh water for either 3, 6, 9 or 12 hours. The heat treatment was done at 70°C for either 30 or 45 seconds. Laboratory trials indicated that freshwater treatment for 12 hours and heat treatment for 40 seconds resulted in high mud worm mortalities. Fresh water treatment reduced Polydora by up to 25 %. Heat treatment was the most successful with Polydora mortality in excess of 30 % with oyster mortality below 15 %.

Lleonart et al (2003) treated *B. knoxi* and *P. hoplura* that heavily infested cultured abalone with a range of chemotherapeutics including Gentian violet (at 5mg.l^{-1}), potassium permanganate (at 15mg/l^{-1}), and mebendazole (at 200 mg.l^{-1}), all treatments were for 3.5 hours at 15 °C. Air-drying was also tested and periods of 3,5 and 8 hours were investigated. Chemical immersion treatment was not highly effective but by contrast the air-drying produced significant effects with up to 50% mortality rate for infesting worms.

Bishop and Hooper (2005) found that treatment of juvenile oysters fourth nightly with a hyper-saline solution (290 g.l^{-1}) for 20 minutes followed by drying in the sun for 2 hours was effective in reducing infestation. This treatment reduced infestation from around 40% to 50 % without affecting shell growth or significantly increasing mortality although treated oysters did show a higher mortality rate.

Large-scale aquaculture operations, especially those in open water will always be at risk of infestation. Land based operations are far less a risk because closer control can be affected. Many of the methods investigated are not practical for large-scale commercial aquaculture that operates under the constraints of expense and available labour. The large scale use of any chemicals should not be considered except for experimental or laboratory work and out of all the methods suggested immersion in saturated salt solutions and air drying would be the least expensive, easiest to use in a commercial production system and least damaging to the environment. These control and treatment methods can be incorporated into the daily operating procedure of the farm especially during grading and sorting operations.

Management of culture systems in estuaries and open water are more problematic but the aquaculturist can still minimized infestation by fairly simple husbandry techniques. Areas where the cultured organisms are exposed to soft and / or muddy sediments especially if resident populations of wild spionids are present should be avoided. The reproductive cycle of any wild spionids should be established and during periods of peak settlement (spring) cultivated animals should be kept as clean as possible and regularly monitored for infestation. Any infestations should be treated with immediate effect. Areas of reduced salinity should be avoided. Exposure to regular intertidal drying will not only improve the quality of the oyster but it will also help to reduce infestation.

Chapter 2 A CASE STUDY OF A POLYDORID INFESTATION ON A SOUTH AFRICAN OYSTER FARM

2.1. Introduction

Worldwide, many economically important bivalve mollusc species are threatened by infestation of spionid polychaetes most often of the genus *Polydora*. Reports in the literature differ in opinion as to the effects of infestations of *Polydora* but in some cases it has been reported to significantly reduce the quality of oysters especially for the live in-shell market. It has also been reported by some authors (Whitelegge, 1890; Roughley, 1922) that such infestations can in some cases lead to massive losses of animals.

In the summer of 2003 Paternoster Oyster Farm situated on the west coast of South Africa, just outside the town of Paternoster experienced such an infestation. The farm also reported large-scale mortalities during the same period. The farm has been in existence for about ten years and has operated with varying levels of success depending on the quality of the management and availability of funds. Two bivalve species have been cultivated successfully on the farm namely the pacific oyster *Crassostrea gigas* and the Chilean scallop *Argopecten purpuratus*. Since the current owners took over the operation only the pacific oyster is cultivated. The farm supplies product into the local live in-shell market and the presence of blisters containing compacted mud and metabolic waste products have significantly impacted on the financial viability of the operation.

Goyard, (1996), observed increases in mortality rates during summer in the Bay of Veys in France since 1994, while Fleury et al. (1999) reported losses of marketable size oysters of as high as 51% in the same area. Although no single cause seemed to be implicated, summer mortality syndrome is probably a combination of several extrinsic and intrinsic factors. Environmental parameters such as temperature, salinity and oxygen and biological factors such as nutrient richness and gonad maturation have all been reported to contribute to massive mortalities in other oyster banks (Lipovsky and Kenneth, 1972; Perdue et al., 1981; Andrews, 1982; Maurer et al., 1986; Soletchnik, 1999; Cheney et. al. 2000). Royer et al. (2005) investigated the possibility that *Polydora* infestation may contribute to mortalities but could find no significant relationship between the prevalence of infestation and mortalities, although it was shown that infestations have a significant negative effect on growth.

This problem of *Polydora* infestation has never before been reported at Paternoster. The study was undertaken to identify the organism that caused the infestation, determine the extent of the infestation and to investigate the impact of the infestation on the condition of the animals.

2.2 Materials and methods

2.2.1 Study Area

Figure 6 An aerial view of Paternoster Oyster Farm showing the three cultivation ponds.

Paternoster Oyster Farm is one of very few land-based oyster-farming operations in South Africa. The farm is situated on the west coast of South Africa, drawing its water from the cold, nutrient rich Benguela current. Cultivation is done in three disused saltpan evaporation ponds approximately 0.5 hectares in size (Figure 6). Seawater is obtained from a set of well points sunk below the low water spring tide (LWST) mark. The well points are approximately two meters below the sand, which consists of course sand and gravel. The water is pumped from the wellpoints and then gravity fed to the cultivation ponds via an open plastic-lined channel. Apart from filtering by the sand the water is not treated any further.

The cultivation is based on a flow-through system. Oysters are reared off-bottom in culture bags placed on either aluminium tables or wire racks (Figure 7). No active management of the water quality and associated fauna and flora is done so no environmental management information was available. The farm has no operational hatchery and the oysters are reared from

10 to 12 mm spat imported from a hatchery in France. The spat is nursery reared using a system of upwellers. (Figure 8).

Figure 7 Aluminium culture tables and wire racks in the cultivation ponds.

Figure 8 Upwellers used for nursery rearing of spat.

Water supplied to the nursery is obtained from one of the cultivation ponds. At a size of approximately 30–40 mm the juvenile are placed in mesh bags and racked in the ponds approximately 0.5 meters above the substrate consisting of sand. Standard cultivation practices for oysters with regards to grading, housekeeping and maintenance are followed at the facility.

2.2.2 Sampling

Samples were taken from each of three different age groups (cohorts) on the farm during the time of the study. The three cohorts were spatially separated with the animals that were approximately one and a half years old (on-farm age) in one pond (Cohort A), a second stocking of one year old animals (on-farm age) (Cohort B) in another pond and the youngest animals (Cohort C) with an on-farm age of 6 months still in the nursery.

The tables (Cohort A) or racks (Cohort B) were numbered and six numbers drawn at random from a hat. Once the tables or racks were selected, the bags were once again numbered and the selection procedure was repeated to select one bag per rack or table. Five oysters were removed in a random fashion from each of the six bags by shaking the bag and then blindly extracting an oyster by hand. In the nursery, six upwellers were also randomly selected by numbering and drawing from a hat and five live oysters were blindly removed from each upwellers. After the selection process the oysters were inspected and any dead oysters were discarded and replaced with a living one from the same bag or upweller.

2.2.3 Determination of condition index

Before the oysters were weighed and shucked they had to be cleaned. The oysters were scrubbed clean with a hard brush to remove any encrusting organisms and then returned to clean, fresh seawater for 1 hour to restore the internal water volume lost during the cleaning process. The oysters were then removed from the water and air dried by placing in a cool shady area for 30 minutes. Any moisture remaining were then removed by patting dry the oysters using paper towels after which they were weighed using a Metler top pan scale to determine the total wet weight (TWW). The oysters were then carefully shucked. Any water in the shells were discarded and the meat (including the adductor muscle) was removed with a scalpel and patted dry with paper towelling and weighed to determine wet meat weight (MW). The empty shells were further air dried for 30 minutes and weighed to determine shell weight (SW). These measurements were then used to determine the condition index (CI) according to a formula described by Aguire, (1979).

$$\mathrm{CI} = \frac{\mathrm{MW}}{(\mathrm{TWW} - \mathrm{SW})} \times 100$$

As a further indication of condition the AFNOR fattening or condition index was determined. The AFNOR index is derived from the French quality norm NF V45-056 (1985), a quality standard implemented by the French to control and determine the quality and condition of cultivated oysters. Oysters are often placed in "claires" or shallow artificial tidal salt marsh ponds for fattening. These ponds are rich in nutrients with consequent high phytoplankton productivity. One species of particular interest is the diatom *Haslea ostrearia*. After death the green pigment from the diatom diffuses into the water and is absorbed by the oyster gills, giving them an attractive dark green colour, much in demand by consumers. An AFNOR index value of between 6.5 and 10.5 qualifies the oysters as "*fine*" or "*fines de claires*" and greater than 10.5 as "*spėciale*" or "*speciales de claires*". Oysters that are kept for more than 4 months at a density of below 5 individuals m^{-2} and with a fattening index > 12 are called "*pousses en claires*". The index is calculated as follows:

$$\text{AFNOR} = \frac{\text{MW}}{(\text{TWW})} \times 100$$

2.2.4 Quantifying the degree of blistering

The inside of both valves of every oyster were inspected with a dissecting microscope for the presence of blisters (Figure 9) and worms to determine the degree of polydorid infestation.

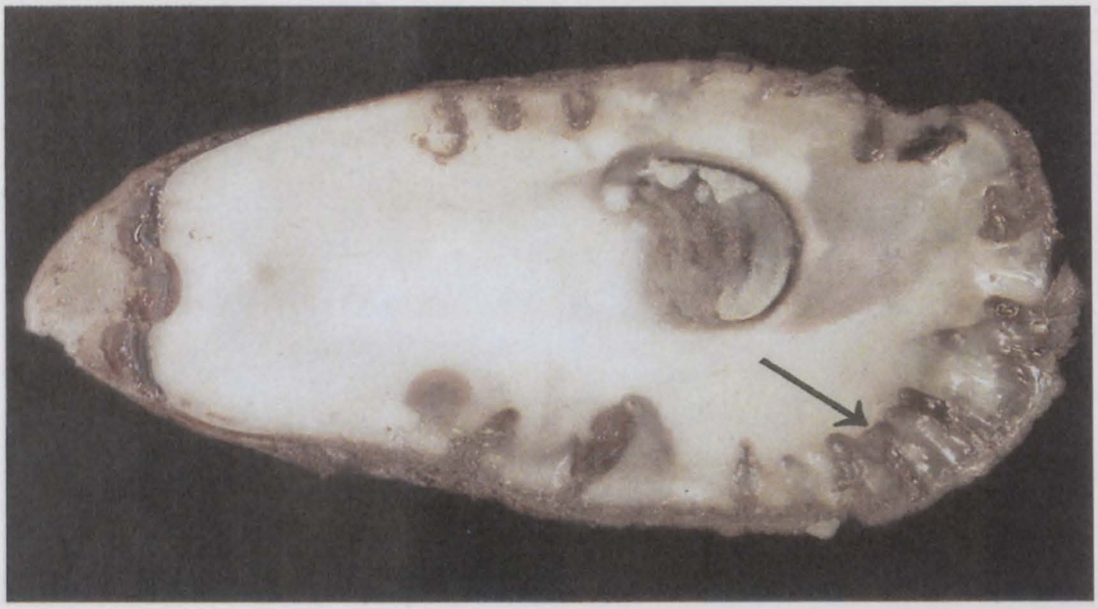

Figure 9 An oyster shell infested by *Polydora* with the U-shaped tubes clearly visible on the margin of the shell.

The tubes were counted and inspected for the presence of live worms. The position of the blisters were also noted and recorded either as marginal (originating on the margins of the shell) or internal. The prevalence of infestation (PI value) is defined as the ratio between the number of occupied and unoccupied blisters and the number of oysters counted, with the PI value calculated as:

$$PI = \frac{n\ \text{Infected}}{n\ \text{Counted}} \times 100$$

2.2.5 Identification of organisms

Six shells with blisters were placed in a clean 1-liter glass beaker, which contained a solution of clean seawater, at room temperature, containing 100 ppm O–dichlorobenzene. As O–dichlorobenzene is difficult to dissolve in seawater, 1 gram was first dissolved in 100 ml of fresh distilled water. 10 ml of this solution was then added to the seawater to give a final concentration of 100 $mg.L^{-1}$ final solution. The shells were exposed to the chemical treatment for a period of three hours. O–dichlorobenzene has been proven to be a very effective vermifuge (Mackenzie and Shearer, 1970), which at levels of 100 ppm cause *Polydora* to emerge but does not cause death. It was important not to cause mortality in the tubes as the count of emerged worms was used to confirm the PI. The first worm appeared after 27 minutes. The worms were transferred to petri-dishes containing fresh clean seawater and left for one hour to recover from the effects of the vermifuge before they were examined under a dissecting microscope and identified to genus level using the key as described in Section 1. Ten oysters were also collected and kept alive in an aquarium for 3 months to make observations on feeding behaviour. All statistical analysis was done using Microsoft Excel "Analysis Toolpak".

2.3 Results

2.3.1 Blisters, degree of infestation and identification

There was a clear difference in the degree of infestation between the cohorts. Cohort A had the heaviest infestation with 60% of the animals inspected found to contain at least 1 polydorid (PI value = 60.0, Table 2). The heaviest single infestation levels were also found on animals in this cohort with at least one animal containing 5 active worms. The animals in this cohort were the oldest animals (18 months) and it is expected that they would be the heaviest infected. Cohort B had a PI of 26.7 and Cohort C showed no sign of infestation. As Cohort C are newly imported animals and still in the nursery it is understandable that they would not be infested yet. Almost all the blisters encountered were on the shell edge with only 2 out of 26 being internal. No more than 5 tubes were observed in any of the infected oysters.

11 U-shaped tubes were identified in the 6 shells that were subjected to the O–dichlorobenzene treatment. All the tubes were well formed and in some worm activity could

be seen. All the blisters were covered with a smooth thick layer of chonciolin and accumulated mud were clearly visible in the tubes. Ten worms were collected during the extraction, which means that at least some of the tubes counted might have been empty. The worms were active and ranged in size from 0.9 – 2.3 cm, with a light reddish brown colour. All the worms were anatomically identical. No individuals smaller that this size were found and as the study was done in May 2004 (the start of winter in South Africa) it is reasonable to assume that the initial infestation took place during spring / summer of 2002. This was also the time when oysters were brought onto the farm from the Knysna Oyster Company for on-growing (Knysna Oyster Company is an oyster farm that cultivated *C. gigas* in the open waters of the Knysna estuary on the south coast of South Africa). The secondary infestation of the second cohort probably took place in the spring / summer of 2003.

Table 2 Prevalence Index (PI value) and the range of numbers of Polydora per oyster. (Number in brackets denotes internal blisters)

	Cohort A	Cohort B	Cohort C
Number Sampled	30	30	30
Number Infected	18	8	0
Prevalence Index (PI)	60.0%	26.7%	0.0%
Animals with 1 blister	5 (1)	5	0
Animals with 2 blisters	6	3	0
Animals with 3 blisters	4 (1)	0	0
Animals with 4 blisters	2	0	0
Animals with 5 blisters	1	0	0

The worms infesting the oysters were identified as spionid polychaetes of the genus *Polydora* using the key provided in Table 1 (Section 1.2). The first 9 Segments were not uniramous and a well-developed rounded prostomium and a peristomium with one pair of long grooved palps was present. The parapodia are well developed. The 5th setiger was clearly enlarged and a row of curved heavy spines was present. Branchiae were absent on first 5 setigers, also started on setiger 6 and continued posteriorly to the second last setiger.

2.3.2 Size and condition of animals

The wet meat percentage is in line with results found for *C. gigas* in the Swartkops estuary (Port Elizabeth, South Africa) by De Keyser (1987) but is significantly lower that those found by Huges–Games (1977) (20%) in Israel and by Fijuya (1970) in Japan (16.6%). The younger oysters seem to have slightly higher percentages of meat which once again conforms with the trend found by De Keyser in Swartkops and Briggs (1978) in Northern Ireland.

Table 3 The average size, level of polydorid infestation, Condition Index (MW/(TWW-SW) x 100) and AFNOR Index (MW/TW x 100) values for 3 cohorts of oysters under cultivation at Paternoster Oyster Farm.

Parameter	**Cohort A**	**Cohort B**	**Cohort C**
Farm Age	18 months	12 months	4 months
Number Sampled	30	30	30
Number Infested	18	8	0
Total Wet Weight (TWW) (g)	45.95 ± 8.43	13.00 ± 5.39	1.80 ± 1.35
Shell Weight (SW) (g)	27.45 ± 5.86	7.59 ± 3.18	0.97 ± 0.78
Shell % (SW/TWW x 100)	59.59 ± 4.23	58.28 ± 4.74	52.44 ± 6.05
Meat Weight (MW) (g)	4.89 ± 1.32	1.94 ± 0.93	0.26 ± 0.19
Wet Meat Percentage (SW/TWW x 100)	11.77 ± 1.70	14.38 ± 1.74	14.91 ± 2.04
Condition Index (CI)	29.43 ± 5.30	35.93 ± 6.83	31.85 ± 5.75
AFNOR Index	11.77 ± 1.70	14.78 ± 2.06	14.91 ± 2.04

The shell percentage is higher that those found for C. gigas in the Swartkops estuary (De Keyser, 1987) but this may be due to the fact that in his study the shells were oven dried for extended periods before weighing. The shell percentage is however in line with the general trends found internationally for the species.

An ANOVA analysis of the condition index of infested versus un-infested oysters show no significant difference between the conditions of the two groups ($F_{1,28}$ = 6.532, P = 0.016 for Cohort A and $F_{1,28}$ = 3,16, P = 0.089 for Cohort B). Further analysis also showed that there was no significant difference between the condition of the infected oysters in the two cohorts ($F_{1,24}$ = 4.208, P = 0.051) and the condition of the non-infected oysters in the two cohorts ($F_{1,32}$ = 6.898, P = 0.016).

Table 4 A comparison of Condition Index (MW/(TWW-SW) x 100) and AFNOR Index (MW/TW x 100) values of polydorid infected versus uninfected oysters found at Paternoster Oyster farm

	Cohort A		**Cohort B**	
	Infested	**Uninfested**	**Infested**	**Uninfested**
Number (n)	**18**	**12**	**8**	**22**
CI	27.57 ± 5.27	32.20 ± 4.13	32.64 ± 6.96	36.96 ± 5.46
AFNOR	12.01 ± 1.41	13.00 ± 1.32	14.75 ± 2.22	14.49 ± 1.59

The AFNOR indexes for all the oysters were high enough (> 10.5) to classify all the oysters as "*fine*" which would make them acceptable for sale.

2.4 Discussion

Identification of infesting organisms

The study was undertaken at the request of the owner of the oyster farm after the presence of "worms" was reported. The farm also suffered severe mortalities in the months prior to the study. *Polydora* was immediately suspected due to the fact that it has been reported to be a problem in oysters in South Africa (De Keyser, 1978). The infesting organism was identified as being a member of the genus *Polydora*. The effects that spionid polydorid infestations can have on oysters have been confusingly portrayed in the literature with some authors (Lunz, 1941; Loosanoff and Engel, 1943; Schleyer, 1991; Stephen, 1978; Caceres-Martinez, *et al.* 1998) reporting no or little impact while others (Korringa, 1951; Handley and Bergquist 1997; Wargo and Ford, 1993) found more significant impacts especially on the condition of the animals. The origin of the infestation must be traced back to the oysters that were brought on to the farm for on- growing from the Knysna Oyster Company. The reason for this assumption is two-fold. The farm gets it water from underground well-points which should effectively filter the water down to at least 100 µm making it impossible for polydorid larvae to enter the system this way. Further support for this is the fact that the well-points are sunk on a long stretch of sandy beach far away from any possible site where natural populations of *Polydora* may be resident. Once the organisms were introduced and became established in the system they would have easily spread from one pond to the other since all the ponds are connected.

The extent of the infestation

The highest prevalence of infestation (PI = 60 %) was found in the oldest oysters (farm age of 18 months). These oysters are also those believed to be responsible for the initial infestation as they were all brought onto the farm from the Knysna Oyster Company. The level of infestation of these oysters prior to their arrival on the farm is however unknown so it is impossible to say whether the high PI is due to a heavy original infestation or secondary infestation at a later date. The second group of oysters showed a far lower prevalence (PI = 27 %), which is consistent with a secondary infestation and the youngest oysters in the nursery were completely free of polydorids. A possible timeline for the progression of the infestation is can be presented as follows. The original infestation took place in the spring of 2002 (September) when the oysters arrived from the Knysna Oyster Company. The second cohort was infested during the next spring (September – December 2003), which is the natural breeding season for polydorids. (Nel, 1996; De Keyser, 1987, Dorsett, 1961; Gudmundsson, 1985; Zajac, 1991). The spread of the larvae to the unaffected oysters was easy as water is fed from one pond to the next. The recruitment period was over when the final cohort was brought into the nursery so no polydorids were found.

The extent of the infestation was far lower (PI_{max} of 60%) than reported by De Keyser (1987), working on oysters farmed in open estuary conditions using the tray cultivation method. He reported heavy infestations (more than 50 % of the shell was covered in blisters) and a PI of 100%. Nel *et. al.* (1996) working with oysters cultivated sub-littoraly using the long-line method in the same geographical area as De Keyser also found polydorid infestations but he does not quantify the prevalence. The silt load in the areas where he found high PI's was far higher than what was found in the current study. It does however support the theory that *Polydora* prefers areas with a heavy silt load (Skeel, 1977). Results found for degree of infestation however is in line with the current study (3.05 polydorids per oyster). Royer *et. al.* reports PI's ranging from 66 – 74% in oyster cultivated in the Bay of Veys in France. Ruellet (2004) reported an average of 40 worms per oyster from the same area. Handley (1995) found a PI of 57% with worm numbers ranging from 4 to 12 per oysters from intertidal rack cultures in Admiralty Bay in New Zealand.

The impact of thePolydora infestation on the condition of the animals

The condition of the oysters was shown to be fairly good (AFNOR index > 10.5, Table 4). The wet meat percentage is in line with results found for *C. gigas* in the Swartkops estuary (Port Elizabeth, South Africa) by De Keyser (1987) and Loosanoff *et al* in Long Island, USA, but is lower that those found by Huges–Games (1977) (20%) in Israel and by Fijuya (1970) in Japan (16.6%). The younger oysters seem to have slightly higher percentages of meat which once again conforms with the trend found by De Keyser in Swartkops and Briggs (1978) in Northern Ireland. The cause for the mortalities could not reasonably ascribed to the infestation of *Polydora*. The resident worms were well established inside the shells and no blisters were found that were not well isolated within the shell. The condition of oyster from the oldest cohort were variable but good in general (CI = 29.7) with the flesh having a good and sweet taste giving an indication that the animals were actually storing glycogen and therefore not under any severe metabolic stress during the time of the study.

2.5 Conclusions

The polydorid infestation provides no clear explanation as a cause of mortality. The observed mortalities cannot be ascribed to the infestation per se but rather to a combination of the infestation and some other factor (most probably a lack of sufficient food during some period prior to the study). As the farm has absolutely no record of water quality parameters (none are recorded) there might however be a multitude of other reasons for the mortality.

The water in the cultivation ponds was fairly clear and lacking sufficient plankton biomass to sustain the animals under cultivation. As this was a short-term study to determine the effect of *Polydora* on condition and to determine the extent of the infestation, no attention was given to other biotic and abiotic factors that might impact on the health of the organisms. It would therefore be reasonable to postulate that the following sequence of events could explain the observed mortalities.

As there are no proactive management of the water quality in respect to the maintenance of adequate phytoplankton density, the animals could have been subjected to a severe shortage of food. If the oysters would also been challenged during this period by an infestation of *Polydora* the animals would have had to channel a large part of their available metabolic resources to the formation of mud blisters. This could lead to a serious resource deficit and could in extreme situations lead to death by starvation. As mortalities are only recorded on a 3-monthly basis, in accordance with the standard grading intervals, it is not possible to determine the exact time, but merely the period during which the mortality has occurred.

The young spat in the nursery receive water directly from one of the cultivation ponds, which expose them to possible infestation. They were however brought onto the farm after the expected breeding season (spring and summer) of *Polydora* and would therefore not be infested as the results have shown. It would however be critical to remove all the oysters that may possibly be infected from the farm before the next *Polydora* breeding season starts or to modify the system in such a way as to isolate the nursery from the rest of the farm. It would not be financially viable to attempt to treat the existing infestation by chemical means. The practice of exposure of the oysters in the nursery to air for at least 3 hours per week have, however, been implemented and no subsequent infestations have been reported although growth seem to have slowed down, due to reduction in food intake. It was recommended that the older oysters (cohorts A and B) should be left in the growing ponds and be cleared out by selling them as they reached marketable size.

It would also be advisable that a proper management system be implemented on the farm to avoid further occurrences of this problem.

Chapter 3 PROPOSED BEST MANAGEMENT PRACTICES (BMP) FOR THE REDUCTION OF POLYCHAETE INFESTATION IN THE CULTURE OF BIVALVES.

3.1 Introduction

To ensure that a high-quality product reaches the consumer several quality assurance systems have been developed, such as certification under an international accepted standard (ISO 9000 series), Total Quality Management (TQM), Best Management Practice (BMP) and Hazard Analysis and Critical Control Points (HACCP). The United States, Canada and the European Union have mandatory HACCP-based seafood regulations. The main aim of HACCP is to identify and assess potential hazards associated with the growing, harvesting, processing, marketing, preparation and use of a food product. This is done by determination of critical control points (CCP's) in the whole process to eliminate or minimize hazard that can be either physical, biological or chemical in nature and to establish systems to monitor CCP's and to take corrective action when a particular CCP is not under control.

Polydorid infestations can have a serious impact on any bivalve cultivation system. It is therefore essential for any such operation to have a management system in place to limit the associated risks. A "Best Practice" can be defined as a process, technique, or innovative use of resources that has a *proven* record of success in providing significant improvement in cost, schedule, quality, performance, safety, environment, or other measurable factors which impact the health of an organization A BMP plan must be practical, make good financial sense, integrate into standard farm practices and effect the desired result.

To effectively construct a BMP plan to control infestations the whole farming process that can possibly be impacted by polydorids is mapped using a process flow diagram (Figure 10). Then using a hazard analysis critical control point (HACCP)-like approach the system can examined to identify areas of possible hazards. Although many believe that HACCP is designed only for use in processing plants, own personal experience have proven that the farming process is just as important to the quality of the final product. European Union HACCP regulations clearly states that fisheries products infested with parasites or fish products showing abnormal odour or flavour (which is the main problem with breaking of mud-blisters caused by *Polydora* infestation) may not be placed on the market. The following BMP plan is proposed for a land based farming system.

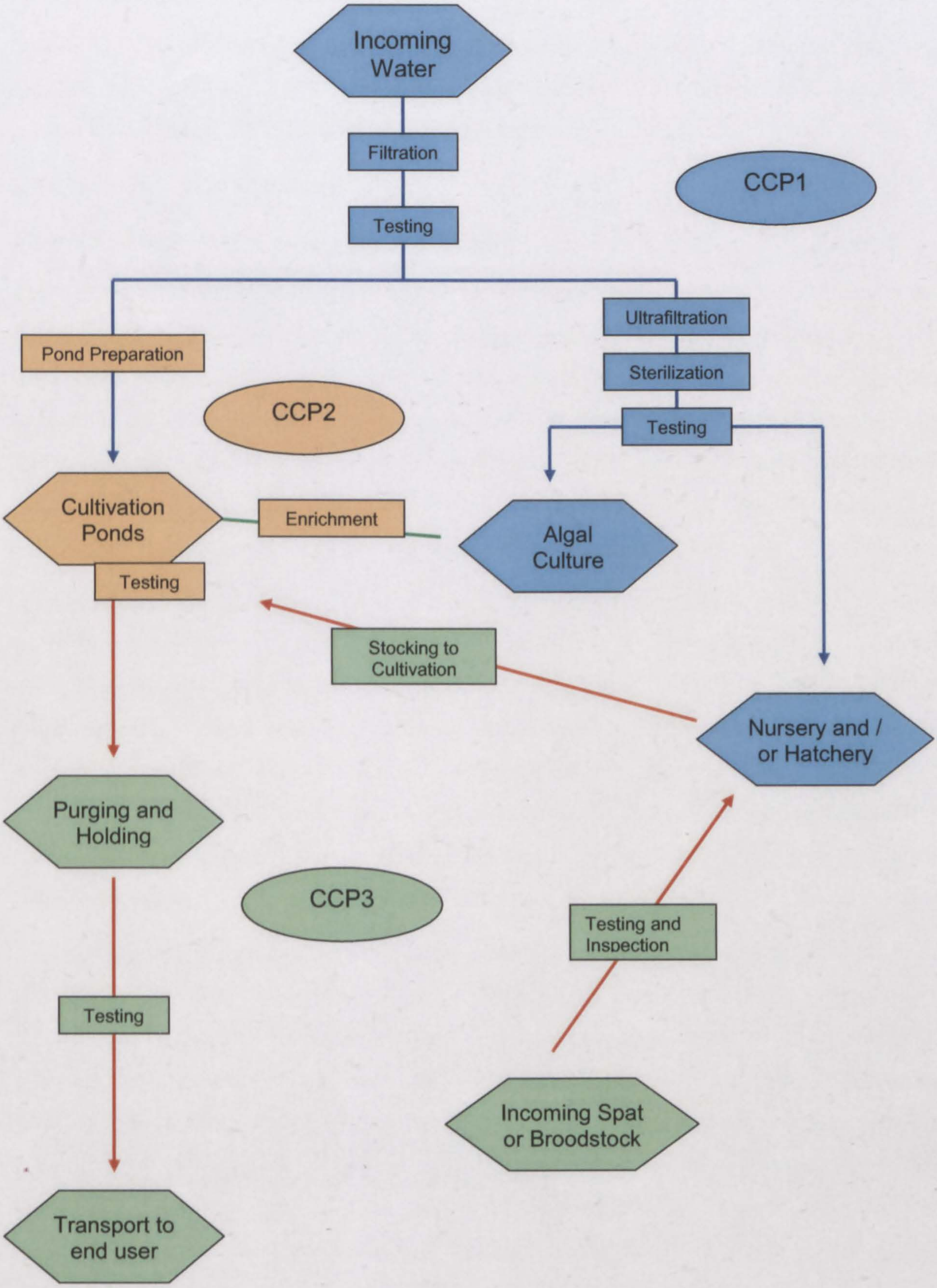

Figure 10. Process Flow Diagram for oyster mariculture.

3.2 Best Management Practice development

To compile a BMP plan it is necessary to clearly understand the process flow of the operation as described in Figure 10. The BMP protocol can be divided into three critical areas of management namely water quality, the cultivation area and the organism.

3.3.1 Management of water quality

Control Point 1. Incoming water quality monitoring

The quality of the incoming water supply to any aquaculture is probably the single most important criterion for success. The water can be supplied to land-based farms in one of two ways. Open pipelines pull water directly from the sea into the farm. This is usually accompanied by rudimentary filtration to remove large particles such as plant matter, fish and invertebrates. The water then needs to move through a whole range of filtration systems to remove smaller particles. These may include sand, drum, low micron cartridge and UV filters depending on the end destination and required water quality standards.

Alternatively well-point systems can be used. These can however be problematic depending on the area where the farm is situated. Access to a beach is obvious critical and depending on the quantity of the water required be a capital-intensive investment. Installation and maintenance is also more labour intensive that open suction lines and beach migration can be a problem in some areas. The benefits of well-points for the control of parasitic and other larvae that can contaminate any system however outweigh any drawbacks. Spionid and other larvae can be prevented from entering the system by using this type of water supply system even if there is a resident population of these organisms in the vicinity of the farm.

From the source high quality water needs to be distributed to various parts of the farm, each with its own set of requirements as to quality. Water supply to cultivation areas is usually in the form of open channels or pipelines, each with their own benefits and hazards. Open channels are very easy to maintain but can have major drawbacks. In a culture system where primary production is critical all attempts should be made to ensure that nutrient rich water reach the system. The growth of marine macro algae in open channels can become a major problem as they removed nutrients from the water. Such channels need to be covered and kept clean which can lead to additional expense. Open channels also expose the water source to other types of contamination such as dust, surface run-off, airborne diseases and human waste.

Closed pipeline systems combined with well-points are probably the best system to use. A well point system acts as a very effective filter and the water is virtually sterile. A closed pipe system will also provide a high quality water feed to areas of the operation (i.e. nursery / hatchery and algal culture) where a supply of uncontaminated water is critical. Drawbacks of a pipeline system can include blockage of pipes by encrusting organisms should they manage to get through the filtration system with concomitant difficulties in cleaning such pipelines. It is advisable to install dual pipelines so that any of the lines can be shut down for maintenance without disrupting water flow.

Most operations have hatcheries and / or nurseries and the water required for this must be of an extremely high quality, requiring the installation of some type of ultra-filtration system that filter to at least 10 µm. A UV filter should also be put in this line to sterilize the water. Hatcheries should have their own independent water supply directly from the source. This is not only important for the successful rearing of spat from egg but also for the culture of algae. An algae culture system is critical for any mariculture operation. Such systems have been well described in the literature (Guillard, 1975, Walne, 1974) and will not be elaborated on here. During the hatchery phase this is the sole source of food for young spat and it is important that the correct range of algal species is fed to optimise growth. During the nursery phase of cultivation young animals should be fed from independent bulk algal culture ponds. The supply of supplementary feeds during this phase in the form of specie specific algae culture is, relative to using natural water, a costly option. It is however more beneficial when considering the significantly improved growth rates that can be achieved. Test done by McCausland (McCausland et. al., 1999) showed that after six weeks of supplemental feeding test animals weighed up to 60 percent heavier than the control. However, the level of primary production in the algal feed ponds will determine the food availability for the spat and should primary production crash or decline the spat will have no food supply and this could lead to high mortalities. The culture of supplemental feed is a time consuming and labour intensive operation but in the long run will benefit the operation greatly.

Very pure, high quality water is also needed for other activities such as purging market ready oysters before sale and emergency response management of nursery animals.

Testing protocols

Water management must take into account the potential for chemical, biological and physical contamination. As incoming water is probably the most critical aspect of any land based aquaculture operation it is also the first. Clear parameters must be set as to the required quality of water that is to be supplied to the farm and every effort must be made to eliminate possible contaminants. A regular regime of testing at source for physical, chemical and biological contamination must be implemented. Special attention must be given to pesticide residues, heavy metal contamination and phytotoxins associated with the occurrence of "red tides". Oysters are filter feeders with the ability to accumulate and concentrate toxins in their body. Water should also be inspected for planktonic and / or benthic larvae such as the polydorids, sabellids and toxic phytoplankton. Other physical water parameters such as salinity, pH, oxygen saturation and temperature should also be recorded. As the water quality required for hatchery / nursery operations are far higher that for cultivation all water entering the hatchery should again be tested and monitored according to set parameters.

Assessment of POF

In the case of Paternoster Oyster Farm the main water supply for the farm comes from a set of slotted PVC well points that have been sunk on the beach around the level of the LWST mark. The well points are approximately 2 meters below the surface. HDPE piping connects the underground well points to a mild steel manifold (Figure 11). The manifold is connected to a pump situated in a pump house from where the water is pumped into a channel that feeds the water to the farm The water is of very good quality and microscopic inspection of samples taken over the period of the study showed no organisms present.

Figure 11 The inlet manifold at the low water level with the pump house in the background.

The open PVC plastic sheeting lined channel runs over a distance of approximately 400 meters (Figure 12). An attempt has been made to cover the channel with black plastic sheet in an attempt to prevent the growth of algae on the lining (Figure 12). This procedure has not been successful and the channel is heavily encrusted with brown and green algae growth. Water flow is very sluggish and areas of stagnant water forms in certain areas. On the farm itself water is fed from the channel to individual culture ponds via culverts (Figure 13).

Figure 12 Channel to farm showing covers and heavy algae growth

Figure 13 Sump showing culvert to pond and a pipe supply to another pond

The nursery has no independent water supply from the beach well point system and water instead is drawn from one of the cultivation ponds and piped to the nursery (Figure 14). The use of culture pond water in hatcheries or nurseries carries with it great risks. Disease can spread very easily from a culture pond and infect the whole nursery leading to the potential loss of all animals. Using water from these ponds without filtering can cause the spread of predators and/or encrusting or otherwise unwanted organisms (i.e. *Polydora*) to the nursery. In the case of Paternoster Oyster Farm it is understandable why this is done. No system have been developed for the cultivation of feed organisms for the developing 8 – 10 mm imported spat in the nursery and spat rely on natural production in the cultivation pond as a sole food supply.

Figure 14 Pump the supply of water from the cultivation ponds to the nursery.

3.3.2 Management of the cultivation area

Control point 2. Pond preparation and water quality management

Primary production ponds should always be prepared very thoroughly before being stocked with animals. In continuous culture systems such as used in oyster cultivation it is advisable to use smaller ponds so that spare ponds are always available should the need arise to move animals or to isolate or eliminate a pond. Pond preparation is also very important. Ponds should be properly cleaned out and allowing it to dry completely before stocking. The bottom of the ponds should be rotovated and limed at a rate of 1 metric tonne per hectare. The lime should then be rotovated into the pond bottom and the bottom flattened. Once all preparations are complete the pond should be filled with clean water and fertilized using 20 kg of triple super phosphate, 10 kg of ammonium nitrate and 15 kg of urea per hectare. Fertilizer should be pre-dissolved in clean water. The water should be inoculated immediately with bulk algae cultures of suitable food species, and if possible aerated with paddlewheel aerators at a rate of 2 hp per hectare. Once algal densities have developed to sufficient levels the ponds can be stocked. Throughout the growing period regular testing of water conditions should be done. Water conditions can be effectively controlled through regular water changes and it is critical that sufficient water is available for this.

Production ponds should never be linked to each other but should operate as isolated independent units. Linking ponds can lead to the spread of any disease and / or contamination that may lead to the loss of all animals on the farm. Each pond should also have an independent water supply. Any problems detected must be addressed immediately. Ponds, in which problems have been identified, must be isolated and treated. If the risk is too high the animals should be destroyed immediately and the pond drained, dried and disinfected. Sanitizing agents such as hydrogen peroxide and calcium hypochlorite can be very effective. Before the pond is used again it must be tested extensively to ensure that the problem is eradicated.

Testing protocols

When ponds are in use, weekly checks of algal density and algal species composition should be done and adjustments made as needed with further inoculation or fertilization or water exchange. The pH levels, oxygen saturation, temperature and salinity should be monitored three times a day. Oxygen levels and pH will be the lowest at early morning before sunrise when, as appose to highest levels of oxygen and pH occurring at midday towards late afternoon. These parameters should be within acceptable ranges during these times and if not adjustments must be made immediately. Testing of the levels of available minerals especially calcium should also be conducted on a monthly basis. Shortages of critical minerals can impact heavily on growth of the organisms in semi-closed systems such as ponds. Accurate records of all testing must be kept for analysis.

Strict criteria have been laid down for the requirements of water for the cultivation of marine molluscs (South African Bureau of Standards, SABS), (Truter, 1994)). Cultivation areas that are found acceptable in terms of their content of metals, pesticides, radioactivity, biotoxicity etc. must be monitored on microbiological grounds over a 10-week period and are then graded. To classify as an "A" grade area 75 percent of all samples examined must comply with the requirement of 300 or less faecal coliforms per 100 grams. Monthly samples must constantly be checked to retain this grading. Standards for discharge of water from the farm is also laid down and need to be complied with to maintain the grading.

Assessment of POF

There is none of the BMP protocols mentioned done on the farm.

Management of the organism

Control point 3. Monitoring and testing of organisms

It is critical that any organism that is brought into the cultivation system is disease and parasite free. Spat and / or broodstock should be quarantined until they have been inspected, tested and certified disease free. If the facilities are not available samples should be sent to competent laboratories to be tested before the animals are integrated into the production system. Suppliers should be audited to ensure that they have the required quality control systems in place. Once animals have been found acceptable they can be integrated into the system. It is however important that animals under cultivation be inspected and tested on a regular basis to ensure their health. Although handling animals is stressful the importance of regular testing far outweighs the impact of such handling. Monitoring of growth is a good method of early detection of problems. Accurate record keeping of all measured parameters will establish general patterns for the farm and can be extremely useful for early detection of problems. During regular grading operations it is also critical that the animals and the bags they are cultivated in be cleaned to remove any sediment, pseudo-faeces and encrusting organisms that will promote the settlement of polydorids (Figure 15).

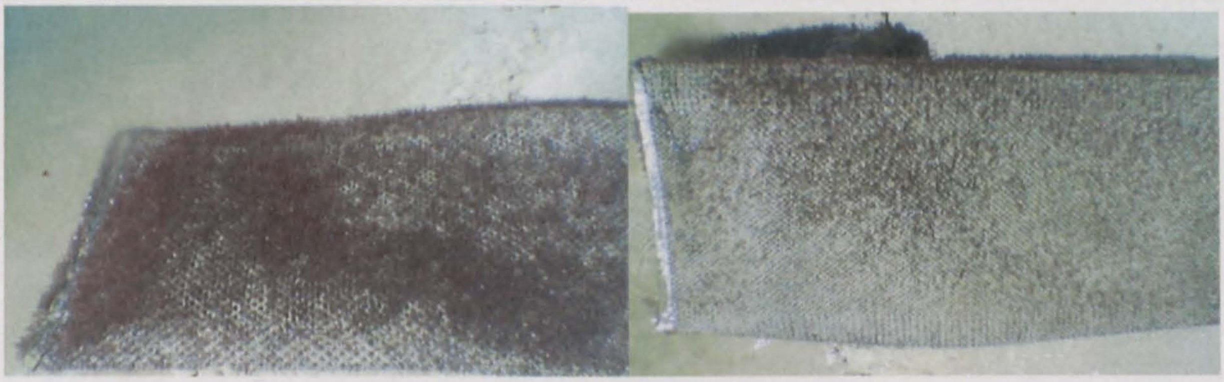

Figure 15 Full oyster bags heavily encrusted with fouling organisms and sediment

Animals that have been selected for market during grading operations must be place in purging tanks for at least three days before packed for marketing. The purging tanks must be supplied with clean, fast flowing, filtered seawater (preferably from the hatchery supply) to ensure that the oysters evacuate their alimentary canals properly. During this period samples of the harvest need to be sent for testing by the SABS (Truter, 1994).

It is advisable that the water is chilled to 5°C to condition the oysters for transport. Chilled oysters should be packed in Styrofoam containers and shipped immediately. Once the cold chain has been established it is important that it is never broken until the oysters reach the consumer. Shellfish must be inspected on arrival by the receiving agent and must be alive (i.e. shells closed) and dead specimens must be discarded.

Testing protocols

The best time for inspection is during grading operations, but in the case of oysters this takes place only every 3 months and these intervals are too long. It is advisable to test samples of animals from every production pond at least once a month. Sampling is done according to statistically significant sampling plan that determines the quantity of animals to be tested according to the batch size. These tests are required by law and should include bacterial testing for faecal coliforms, *Salmonella* and *Vibrio*, chemical testing for pesticides, petrochemical, organohalogenated substances, heavy metal contamination and biotoxins (PSP, DSP and ASP). All of the test results should be negative for marketing and distribution to commence.

Monthly monitoring of growth of individual batches in the cultivation ponds should also be done. Whole wet weight, shell length and weight (dry), wet meat weight and dry meat weight should be determined as these are the parameters used to determine the condition index index of the animals. Reproductive condition should also be noted. During this the animals must also be inspected for the presence of parasites and other indicators of disease and immediate corrective action taken. All data must however be sensibly interpreted in conjunction with environmental parameters to establish long-term trends for the operation.

Assessment of POF

Animals that are market ready are tested as per regulations. Purging is done, but again cultivation water is used and this therefore becomes a meaningless exercise. No scientific monitoring of growth or condition is done, but animals are weighed when they approach marketable size.

Grading is done but the condition of many of the bags with regards to fouling was shocking and there is a clear lack of housekeeping on the farm.

3.3 Conclusion

The implementation of a BMP protocol is critical for the successful operation of any aquaculture venture. It is very clear that the total absence of such a system is the major, if not the only contributing factor to the problems experiences at Paternoster Oyster Farm. Had such a system been in place the problems that was experienced would have been identified early and could have been addressed timeously. If such a system is not implemented immediately it is a certainty that the future success of the operation is in serious doubt.

Ps. Since the completion of the study at the end of 2004, it has been reported that although the problem of *Polydora* was rectified the company continued to experience large mortalities into 2005 and that operations were terminated towards the end of that year and the farm put up for sale.

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