Chapter 2
Host–Symbiont Relationships: Understanding the Change from Guest to Pest

Robin M. Overstreet and Jeffrey M. Lotz

Abstract The several meanings for the term "symbiosis" create confusion, which can be avoided when the author provides details of the interrelationships between the symbiotic organism and the "host" so that a reader can clearly understand what definition is implied in each case. For example, we, as opposed to many other mentioned readers, consider a symbiont as an organism living in an association with another regardless of whether it causes a pathologic response or not, but from our title, the reader may incorrectly infer that we consider a parasite to be different from a symbiont. A symbiont is an organism that uses another organism as a habitat. This chapter discusses the primary associations and associated conflicts involving the terminology. It also provides both differentiation between and conflicting views regarding the interpretation of the terms "infect" and "infest," "infection" and "disease," and other terms. Many seemingly harmless symbionts of a wide array of taxonomic groups are triggered to become pathogenic or virulent, and we provide several examples of the provoking (stimulating) triggers, with the understanding that in most cases, the conditions for the triggered activities are much more complex and complicated than presented. Examples of triggers follow: environmental ones like temperature, toxic chemicals (dose), chemotherapeutics, dietary changes, and geographic habits; internal ones like host site, host resistance or susceptibility, and host modifications; and combinations of these and other conditions. We provide examples involving multiple triggers for organisms associated with termites, for an endemic virus being affected by multiple factors and having multiple effects on its commercial penaeid shrimp hosts, and for contrasting variables associated with two exotic viruses in wild and cultured commercial penaeid shrimps with an emphasis on hypothesizing how the pathogenicity developed in these two viruses. The chapter ends by trying to answer the question of why would a symbiont become pathogenic in some hosts and not in others from an evolutionary perspective. It uses two hypotheses to explain the increased virulence.

R.M. Overstreet (✉) • J.M. Lotz
The University of Southern Mississippi, 703 East Beach Drive, Ocean Springs, MS 39564, USA
e-mail: Robin.Overstreet@usm.edu

C.J. Hurst (ed.), *The Rasputin Effect: When Commensals and Symbionts Become Parasitic*, Advances in Environmental Microbiology 3,
DOI 10.1007/978-3-319-28170-4_2

2.1 Introduction

When reading an article on symbiosis, most readers assume they know the definition of all the associated words in the document. This is not the case; even the term "symbiosis" is defined differently by different authors in different fields, by those in different geographic areas, and by those taught by different mentors. The term "symbiosis" was originally used by the German de Bary (1879) to mean "living together." His meaning referred to all situations where either similar or unlike organisms or species live together in an intimate association. This is typically thought to include "commensalism," "mutualism," and "parasitism." We prefer to include all such associations under the general term "symbiosis" but realize that our title does not comply, and many readers prefer to use a more specific term for symbiosis and other associations. In the preface of his book (1970), the eminent parasitologist William Trager quoted "the conflict in nature between dissimilar kinds of organisms has been popularly expressed in phrases like 'struggle for existence' and 'survival of the fittest.' Yet few people realize that mutual cooperation between different kinds of organisms—symbiosis—is just as important, and that the 'fittest' may be the one that most helps another to survive."

For purposes of this series, we restrict "symbiont" to organisms that use other organisms as habitat. Therefore, symbiont is a division of nature similar to "terrestrial" or "marine." The symbiont is characterized by where it lives rather than the quality of the relationship. For our purposes, we divide up the world into two kinds of organisms, free living and not free living. This accords us the advantage of comparing the quality of the relationship between free-living organisms and their habitat with that between host and symbiont. The symbiont is dependent upon the host as any inhabitant is on its habitat. We consider symbionts to include bacteriophages (viruses that infect bacteria), bacteria, viruses, "protozoans," and metazoans and symbiosis to include commensalism, mutualism, parasitism, and other relationships. Moreover, all symbionts, even metazoans, observed best with a microscope will be considered microbes. More important, we will examine in this series a shift in the interaction among a host and some stage in the life cycle of the symbiont, whether it be free living, facultative, or obligate. We will emphasize that some symbiotic relationships can change from harmless to something that becomes far less favorable for the host.

2.2 Definitions

Symbiosis This term as defined above is usually thought of as an association (mutualism, commensalism, or parasitism) between organisms of different species involving a unilateral or bilateral exchange of material or energy. The symbiont, or symbiote, is any member of a pair of organisms involved in this symbiotic relationship, with the larger member usually designated as the host. Barrows (2011)

provided a classification of symbiosis separating nonsocial symbiosis from social symbiosis, and others classify various terms differently. Most of these terms and classifications will not be treated in this chapter.

Commensalism A symbiotic relationship in which one of two partner species benefits and the other shows no apparent beneficial or harmful effect.

Mutualism A symbiotic relationship in which two or more partners gain reciprocal benefits, usually mutual ones.

Parasitism A symbiotic relationship in which a symbiont lives all or part of its life in or on a living host, usually benefiting while harming the host in some way and usually having a higher reproductive potential than the host. Noble et al. (1989) define it as an association between two different species of organisms in which the dependence of the parasite on its host is metabolic and involves mutual exchange of substances; this dependence is the result of a loss of genetic information by the parasite. There are several atypical kinds of parasites. An accidental one infects an unusual or unnatural host; a commensal one derives its substances from the food of its host; and an erratic, or aberrant, one infects an unusual site. A facultative parasite is usually parasitic but is capable of an independent existence, while an obligate one cannot lead an independent, nonparasitic existence. The late Gerald Schmidt's definitions are listed by Roberts and Janovy (2009). He considered a parasite the "raison d'être" for parasitologists, a parasitologist as a quaint person who seeks truth in strange places and one who sits on one stool while staring at another, and parasitology as a study of the most common mode of life on earth. Many parasites have complicated life cycles with multiple hosts and with a variety of stages, not all of which harm or depend on the host. One can glean that students find it difficult to define a parasite. This series will treat a variety of facultative and other relationships.

Predatory association A relationship in which a predator obtains its living by preying on other animals, usually consuming all or part of it, usually killing it. This relationship can be symbiotic when a predator feeds on one or a few species or when it is relatively small and not harming its host. For example, a trematode (all trematodes are considered parasites) may live in the lumen of a host without causing any harm; that trematode is occasionally called a micropredator.

Other terms that will be causing confusion in this series are *infection* and *infestation*. American parasitologists usually define an internal association as an infection, whether harm is caused or not or the organism is small or large. If the association is external, it is referred to as an infestation, and the symbiont is called an ectosymbiont or an ectoparasite. For an internal association to be called an infection, microbiologists usually restrict the agents to viruses, bacteria, and fungi. Consequently, some authors restrict an infestation to a metazoan parasite (e.g., Maggenti et al. 2005). Barrows (2011) provided three meanings for an infestation: (1) a parasite's colonization, utilization, or both of the host; (2) a host being colonized, utilized, or both by parasites; and (3) and environment being colonized,

utilized, or both by pests. Other authors consider an infestation to refer to a population rather than to individuals. Still, others use the word "infestation" to suggest an action and the term "infection" to suggest a condition or a state. Microorganisms such as oral bacteria that live naturally in the mouth or elsewhere in a body are not considered infections or infectious agents by many microbiologists, but the organisms are symbionts.

Another important term for this series is *disease*. In medical cases, this often refers to dose. A well-nourished person infected with the American hookworm (*Necator americanus*) with a normal hemoglobin and five eggs per milligram of feces, perhaps resulting from up to 50 adult worms, has an "infection" but without a corresponding disease caused by the loss of blood or other detrimental signs. An egg count of 20 per mg in a healthy person or 5 in one anemic with iron or protein deficiency usually indicates the threshold of disease. Massive infections with disease can produce as many as 50 eggs per mg of feces (Beaver et al. 1984). Lightner and Redman (1998) discussed this term when dealing with shrimp infections. They said "In veterinary and human pathology, the terms 'disease' and 'syndrome' have a range of definitions. Dorland's Medical Dictionary (1968) defines disease as 'a definite morbid process, often with a characteristic train of symptoms' and syndrome as 'a combination of symptoms [signs] resulting from a single cause or so commonly occurring together as to constitute a distinct clinical entity.' A simpler definition of disease is 'any alteration from the normal state of health.' The latter definition permits the inclusion of alterations in health that may result in subtle conditions in which poor health or reduced resistance to stress are the only signs of disease, as well as those disease syndromes on the other extreme, that may be accompanied by catastrophic losses."

An important, prudent course of action for an author is to carefully define a term or description that is being treated in any manuscript. A reader, on the other hand, may have serious problems understanding what is being said without that definition. Terms being used in symbiosis are especially difficult because research conducted to define an entire relationship seldom exists. Several glossaries are careful not to include definitions of either infections or infestations, and others are careful not to define a parasite! Many terms used for defining ecology of parasites such as "incidence," "prevalence," "intensity," "mean intensity," "component population," and many others are used differently by different people, and we strongly recommend the article by Bush et al. (1997) as a standard or at least a source of discussion.

2.3 Factors Triggering a Harmful Relationship

Usually, a combination of factors triggers a harmful condition, but only one or two of these factors are known in any detail for most cases. Furthermore, most of the examples below treat only one or two of these triggering factors. Differentiating the triggering factors into those from the external environment and those originating from the host is, for the most part, impossible to characterize because the two are

typically entwined. Moreover, genetic triggers that involve expression such as upregulation, downregulation, and gene knockdown may each necessitate a variety of triggers for expression. Triggers causing a harmless symbiont to transform into a harmful one can also involve the initiation, interruption, or inhibition of biochemical pathways, complicated actions that sometimes incorporate a cascade of reactions. This chapter will not treat the details of mechanisms but rather present examples of what appear like results of cause-and-effect. As indicated above, the headings indicate only some of the triggers in each case, with an emphasis on the primary one.

2.3.1 Environmental

2.3.1.1 Temperature

Temperature often regulates disease in marine animals to benefit stocks of all parties. For example, the English sole, *Parophrys vetulus*, arrives into its estuarine nursery area in the northern Oregon Coast from January to April, grows dramatically in the upper estuary during the summer, and then migrates offshore in the autumn, never to return inshore. When inshore, the sole gets infected by the microsporidian *Glugea stephani* that develops in the intestine at temperatures $\geq$15 °C. This agent appears to cause mortalities from September through November, but this period is when most of the fish routinely migrate offshore where the temperature remains less than 10 °C, a temperature that was shown experimentally to inhibit the microsporidian agent's development, which ultimately results in uninfective spores within a few months. Consequently, the parasite seldom harms the host stock. However, approximately 10 % of the sole stock remained in the upper estuary where an estimated 80 % of those fish had a rapidly developing infection and most probably died and decomposed or were eaten, dispersing the spores. Those fish plus the overwintering infected fish and few reservoir starry flounder juveniles (*Platichthys stellatus*) (a reservoir host is a primary host that harbors the pathogen but typically exhibits no ill effect and serves as a source of infection) provided infective spores for next year-class of sole (Olson 1981; Overstreet 1982).

Temperature also has a great bearing on many other pathogenic triggers. For example, the coccidian *Calyptospora funduli* commonly infects the liver, pancreas, and occasionally other tissues in the Gulf killifish, *Fundulus grandis*, in the coastal Gulf of Mexico. The fish becomes infected by feeding on either the infected intermediate host *Palaemonetes pugio* (the common daggerblade grass shrimp) or related species. Infections are synchronous, meaning that all the developing stages are the same. Early developing stages and mature oocyst stages are rarely seen in the same fish. In some cases, over 95 % of the liver can be infected without causing any notable severe harm to the host. When heavily infected and lightly or noninfected killifish were maintained in outdoor raceways and freezing occurred,

the heavily infected fish all selectively died. Presumably, that was because the liver serves as a storage reservoir for necessary glycogen, vitamins, minerals, and other necessary nutritional resources. Those resources found in 5–10 % of the liver were presumably not enough to satisfy the needs of the fish during stressful low-temperature conditions. A series of experimental infections conducted at about 22, 10, and 7 °C for periods between 5 and 20 days in length (Solangi et al. 1982) showed that both low-temperature treatments inhibited all developmental stages. When the fish were returned from the low temperatures to 22 °C, development of all the stages resumed except in fish exposed to 7 °C for 20 days. In those infections, many of the coccidian organisms were atrophied or disintegrated within their parasitophorous vacuoles, and necrosis also occurred in some of the pancreatic tissue. In any event, decreased parasitism during inhibition was not linked with a leucocytic inflammatory response. When fish remained at a constant temperature of about 24 °C, an inflammatory response to the organism commenced at day 18, intensified at day 20, and diminished by about day 30 (Hawkins et al. 1981, Solangi and Overstreet 1980). In other words, inflammation associated with infections in warm water begins as gamonts developed and ends after formation of the oocyst wall. Consequently, infections that occur during cold winters can be either helpful or harmful to the host, depending on the stage of the parasite, the extent of low-temperature exposure, the age of the fish, and other variables.

Atypical temperatures, such as warm water associated with power plants, can cause infections of a specific parasite during periods when the hosts are more likely to be consumed by predators, more susceptible to disease, or more susceptible to interactions among parasites that can occur and result in unusual pathogenic conditions.

As also suggested above, temperature can have an effect on development of a parasite and can occasionally result in a harmful effect. The ascaridoid nematode *Contracaecum multipapillatum* in a coastal lagoon at Celestún, State of Yucatán, Mexico, infects as definitive hosts the olivaceous cormorant and the great egret. The intermediate host for this avian nematode is the Mayan cichlid (*Cichlasoma urophthalmus*). The juvenile in the fish host for all members of the genus *Contracaecum* is usually a third stage; however, in the warm area of Mexico, many of the juveniles had developed into the fourth stage. When these fourth-stage juveniles were fed to a kitten, they developed into adults in the intestine, often associated with hemorrhaging small ulcers (Vidal-Martínez et al. 1994); they did not develop or cause a pathogenic response in rats, ducks, or chickens. People commonly eat the cichlid in Mexico and, consequently, are potential hosts. When Deardorff and Overstreet (1980b) fed third-stage juveniles, the typical stage occurring in fish, to day-old chicks and ducklings and to mammals, they neither developed nor survived; however, when the third-stage juvenile was surgically inserted within tied off semipermeable dialysis tubing into the abdominal cavity of the animals, the worms did mature.

The gnathostomatid nematode *Echinocephalus sinensis* matures in the intestine of the eagle ray, which acquires the third-stage juveniles by eating the infected oyster *Crassostrea gigas*. Infections in this commercial oyster were most prevalent

and had the highest intensity in Hong Kong between July and September. Kittens, monkeys, and puppies were fed the infective stage from oysters during every month, but those worms collected only from the warm months of August to October infected the mammals (Ko 1976). The worms penetrated the wall of the stomach and intestine, migrated to and lodged in various tissues, and often killed the hosts. To test this temperature-triggering condition, Ko (1977) obtained oysters during the infective season and acclimated them to 5, 15, about 24, 28, and 33 °C. Worms from these different temperature groups were fed to kittens, and infections were most abundant in the 33 °C group and were less so in the 28 °C group; only one kitten became infected from the 24 °C group, and no kitten from the 5 or 15 °C group became infected. Since people eat raw oysters, the nematode constitutes a potential public health risk.

2.3.1.2 Environmental Habitat

Under normal environmental conditions, parasites seldom harm their hosts. When the habitat is a series of aquaculture ponds, several conditions are modified. The pond design usually accommodates a heavy density of snail intermediate hosts near the shallow shore where fish fry occur, and the fish stock inhabiting the middle of the ponds creates an abundance of prey for fish-eating birds. For example, primarily in Mississippi but also less so in adjacent states, the channel catfish, *Ictalurus punctatus*, is reared, comprising a multimillion dollar industry. In the natural environment, catfishes, including their madtom host relatives, do not occur as juveniles in habitats where the host marsh ramshorn snail, *Planorbella trivolvis*, and American white pelican, *Pelecanus erythrorhynchos*, occur and defecate in any abundance. Consequently, infections of the metacercaria (larval stage acquired from a cercaria shed by the ramshorn snail) of the pathogenic diplostomoid trematode *Bolbophorus damnificus* are relatively rare in those natural waters. A total of five different diplostomoid trematode species infecting the catfish were able to kill it in aquaculture environments. Two of these developed from cercariae shed from *P. trivolvis* that ultimately mature in the American white pelican; two were from worms shed from the ash gyro, *Gyraulus parvus*, that mature in the double-crested cormorant; and the fifth species is one that matures in seagulls. The most harmful species, *B. damnificus*, can be present in the catfish with infections of less than 40 metacercariae without harming it. Heavier infections seem to have an effect on the kidney of the fish aiding in their mortalities. In the shallow water where large numbers of infected snails occur, thousands of cercariae can penetrate the young catfish and kill it quickly, even before developing into metacercariae. This is in contrast to two other trematodes, *Bursacetabulus pelecanus* and *Austrodiplostomum compactum*, which both infected the nerve cord, brain, optic nerve, and eye and at least in experimental infections can occur as several hundred individuals without killing the host (Overstreet and Curran 2004). The trigger for mortality regarding *B. damnificus* can be more complicated than pure numbers. In experimental infections with fingerling channel catfish exposed to a sublethal

infection of trematode cercariae per fish, they died when additionally exposed to the bacterium *Edwardsiella ictaluri*, the agent of enteric septicemia of catfish. Controls given either the cercariae or nothing did not die. Those given 7.5×10^5 colony-forming units per mL of bacteria for 30 min singularly without cercarial exposure started dying at day 7, and, by day 21, the percent cumulative mortality leveled at about 46 compared with 84 % for the group given both the cercariae and bacteria. Then at day 28 when the metacercariae fully developed, groups consisting of the remaining fish given only the trematode and of the negative controls were exposed to the bacteria. Both groups starting dying at day 7, but, by day 21, there was no significant difference in the percent cumulative mortality of about 18 % (Labrie et al. 2004). When contaminating forage fish that occur in the ponds in addition to the commercial catfish became exposed to the cercariae of *B. damnificus*, they did not become infected. On the other hand, there is an unnamed species of *Bolbophorus* which also infects both the same snail and pelican, concurrently with *B. damnificus*. However, it does not use the catfish as the second intermediate host, but it uses mosquitofish and sunfish, which it can kill when in high numbers (Overstreet et al. 2002). Also of note is the finding that channel catfish exposed to the *E. ictaluri* bacterium 1 day prior to being exposed to the pathogenic ciliate *Ichthyophthirius multifiliis* had 71 % mortality compared with 27, 29, and 0 % for those respectively given only the bacterium or the ciliate or given neither. The bacterium could be detected by PCR in the gills, brain, liver, and kidney of the fish whether infected with the ciliate or not, but, by day 8, the bacterium no longer persisted in the bacteria-only group except in the kidney (Shoemaker et al. 2012).

A few free-living amebas are well known because they can infect humans and occasionally cause fatalities (see Overstreet 2013). Best known is *Naegleria fowleri* because it causes fatal "primary amebic meningoencephalitis (PAM)," but it is restricted to freshwater, and victims are typically those who swim for extended periods underwater in open bodies with a silty-muddy substratum. The swimmers' nasal mucosa becomes weakened to the point that the ameba can penetrate the membrane and enter the central nervous system (CNS) along the olfactory nerve. Because that site contains little cellular inflammatory response but provides a good medium for amebic growth, the organism replicates rapidly, usually before the disease can be diagnosed and treated. Marine free-living species of the genus *Acanthamoeba* do not grow as rapidly and, depending on the species, invade different sites. They enter the skin, lower respiratory tract, or nasopharynx, and the vegetative trophozoite can reach the CNS through the circulatory system. These acanthamebic infections may take weeks or months to cause death, and often infections occur in patients that are immunocompromised (unlike the otherwise healthy hosts that become ill due to PAM) but are still difficult to diagnose. Six different ameba species have also been associated with painful amebic keratitis, a difficult to treat corneal disease. Even though recognized in 1973, amebic keratitis was not common until 1985 when contact lenses became popular; these lenses typically are maintained overnight in a saline solution that can become a source of contamination.

2.3.1.3 Chemical

Toxic waste products can trigger a reduction in host resistance, resulting in susceptibility to rapidly reproducing agents that would normally be held in check or not able to produce infections. The myxosporidian *Henneguya gambusi* infects the western mosquitofish, *Gambusia affinis*, and is rare, not previously considered pathogenic, and not known from any other fish (Parker et al. 1971). We have also seen it in mosquitofish but only from a location in Mississippi receiving wastes from a timber treatment facility, involving the wood preservative chromated copper arsenate, a mixture of chromium, copper, and arsenic (as copper(II) arsenate) (Overstreet 1997). Presumably, the mosquitofish becomes infected from being in close contact with actinospores shed from a tubificid oligochaete. A small percentage of the few fish in the contaminated creek exhibited mass infection (Figs. 2.1 and 2.2), and it was typically histozoic with plasmodia throughout the skeletal muscle mixed with tissue debris not associated with an obvious inflammatory response rather than in pseudocysts located in the epidermis, corium, and subdermal connective tissue as originally described. A few of those fish from the wild had a severely pathogenic infection, with mature spores liberated from the plasmodia and spread into adjacent muscles, replacing most tissues throughout the body (e.g., Dyková and Lom 2007). However, when we collected 50 fish from this station and 50 from another station containing tubificids and then maintained them in aquaria in our laboratory and fed them commercial flakes daily, over half the fish from the contaminated location became moribund or died within 2 months. No fish from the other location died or appeared unhealthy. The moribund fish were sectioned or examined fresh and demonstrated the myxosporidian with its 10 by 6 μm spores

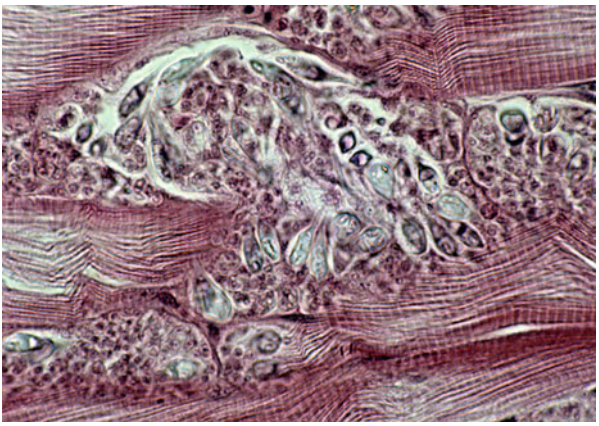

Fig. 2.1 Mass infection of the myxosporidian *Henneguya gambusi* triggered within the skeletal muscle of the western mosquitofish, *Gambusia affinis*, from a location in coastal Mississippi that contains heavy metal contamination. Vegetative and mature stages in plasmodia located between and within muscle fibers, with mature triggered spores being liberated and destroying muscle and showing no plasmalemma separating spores from fibers

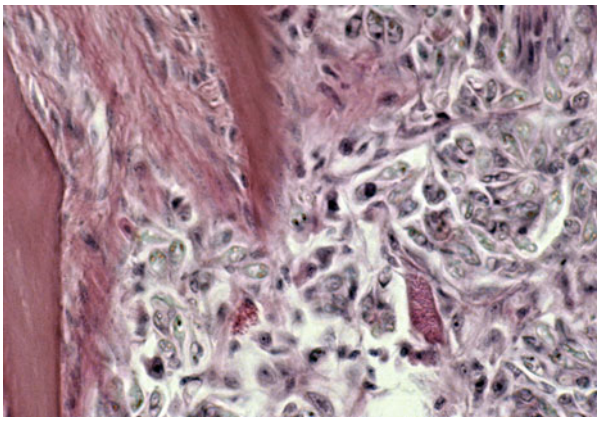

Fig. 2.2 *Henneguya gambusi* in *Gambusia affinis*. Mature spores with some liberated ones from plasmodia leaving only remnants of muscle fibers. Note lack of host inflammatory cells

invading most tissues throughout the samples. Representatives of live fish from both groups did not exhibit infection in section, and none of the remaining fish exhibited infection when critically examined as fresh. The contamination apparently reduced the host's protective immune response which would normally keep a low infection in check. Light infections had to be present in about half the fish from the contaminated location when brought into the laboratory, even though not evident in fresh or sectioned material. Under normal conditions, spores of all histozoic species occur in small to large pseudocysts, the latter containing millions of spores. When the plasmodium is extremely small or when few vegetative or spore individuals are present, an infection is difficult to see, and polymerase chain reaction (PCR) probes are necessary to detect infections. In fact, the only person we have seen skilled enough to routinely detect minimal infections is Iva Dyková of the Academy of Sciences of the Czech Republic.

Another example of a pathogenic agent in the western mosquitofish deals with a free-living organism rather than a known parasite. The free-living organism, the ciliate *Tetrahymena corlissi*, has been reported from fish in aquaria and hatcheries, causing pathological alterations and mortality by Hoffman et al. (1975). They were unable to experimentally infect fish with the ciliate and suggested that infection resulted from a wound or stress. We (Overstreet et al. 1995) sampled the mosquitofish from an outlet canal from an integrated pulp and paper mill, 2.5 km upriver from the canal where water entering from a dam kept mill effluent from flowing upstream, and three downstream locations. Two of 99 fish from upstream, but none from the other locations, had a proliferation of the ciliate in the head and in the musculature, brachial chamber, and pericardial sac; that location also had fish with a significantly higher prevalence of macrophage aggregates in the spleen, a good indicator of stress. After publication, we learned of a long submerged pipe emptying toxic wastes upstream from our locations and originating from a nonrelated facility located several km from the river and apparently promoting the ciliate infection. Some ciliate specimens measured larger than those reported by Hoffman et al. (1975), but sequencing of presumed free-living ciliates infecting

various aquatic hosts should allow identifications and experimentation to determine details of the mechanisms that shift a free-living organism to become a pathogenic one. For parasites or diseases of a host organism to be used as monitors of environmental health or biological activities, both the host and the symbionts or pathological responses need to fit several criteria (Overstreet 1997).

Chemotherapeutics can also serve as toxins. A CDC report (U.S. Department of Health and Human Services, Centers for Disease Control and Prevention 2013) estimated that about 100 million people worldwide possess a chronic infection with the nematode *Strongyloides stercoralis*, and prevalence of the infection in refugee populations ranged from 11 to 69 % by serosurveys. This species has the unusual ability to replicate and auto-infect its human host, persisting for decades. *Strongyloides* hyperinfection syndrome may be triggered many years after migration of a prior refugee to a nonendemic locality, with large numbers of the parasite infiltrating internal organs, resulting in fatality rates exceeding 50 %. The syndrome is generally induced when an individual is placed on corticosteroids, although other immunosuppressive conditions such as cancer and transplant chemotherapeutic immunosuppression may also trigger the hyperinfection.

A change in diet as well as some chemotherapeutic compounds can serve as a trigger and induce a parasite located in one internal habitat such as the intestine, blood, visceral organ, or muscle to migrate to more sensitive tissue or be released from a cyst or an encapsulation and migrate or replicate.

2.3.2 Site Within Host

Free-living or symbiotic metazoans, protozoans, algae, fungi, and bacteria can get into abnormal sites (locations in host) or habitats and develop into pathological agents. Some cases are rare and others are common. A rare case consisted of finding the diatom *Amphora* sp., which normally is a free-living alga, in the white shrimp, *Litopenaeus setiferus*, presumably after the shrimp's carapace had been punctured or abraded. The diatom occurred in abundance as clusters in the hemolymph, plus some individuals were present in the gills associated with a melanistic response. The shrimp died. To test the effect of the related *Amphora coffeaeformis* on shrimp, Overstreet and Safford (1980) injected cultures into shrimp resulting in melanistic responses, but no extensive replication occurred as had been observed in the shrimp killed with *Amphora* sp. A more common opportunist is the fungus *Fusarium solani*, which also developed in penaeid shrimp. *Farfantepenaeus californiensis*, a species of penaeid initially being considered for aquaculture, is a highly susceptible species to this fungus. When experimentally infected with a cultured conidial suspension of this free-living species (Hose et al. 1984), all shrimp became infected within 14 days, tissue destruction and a strong but often unsuccessful hemocytic inflammatory response occurred, and over half of the shrimp died within 24 days. Biochemical and hemocytic parameters of the hemolymph changed significantly as the infection developed when compared with noninfected shrimp. When *Farfantepenaeus aztecus* or *Litopenaeus setiferus* was held in seawater with

macroconidia spores cultured from an infection from *Farfantepenaeus californiensis* or injected with those spores, resistance to infection occurred. A strong hemocytic encapsulation and melanization of the macroconidia and lysis of the conidiospores occurred in the gills by 28 h. If spore dosage was excessive, 3.2×10^6 or greater, all brown shrimp died within 24 h from the macroconidia and hyphae blocking the distal portion of the gill lamellae (Solangi and Lightner 1976). In *Litopenaeus vannamei*, the primary cultured penaeid today, naturally infected individuals in culture are moderately susceptible to infection and the spore aggregate, often grossly apparent in the distal portion of the eyestocks; *Penaeus monodon*, which used to be the primary commercially cultured species, is relatively resistant (Lightner 1996). For pathogenicity by free-living algae and fungi, the triggering factor seems to be a wound or being compromised by other infectious agents or toxicants, but, as pointed out, the host species is also critical.

One or more ciliates identified as or presumed to be the scuticociliate *Orchitophrya stellarum* has an extensive host and geographic range in Europe, Australia, and North America as determined by morphological and sometimes molecular techniques. It is considered a facultative parasite that can live indefinitely outside the host when cultured with bacteria and tissue detritus or yeast but with different size and morphology when compared with material in male sea stars. Cultured specimens enter male but not female sea stars, probably through the gonopores, where it enters the testes and feeds on sperm of fully mature individuals only (Stickle et al. 2007). It has been reported from several sea star species and may have been inadvertently introduced into the Northeast Pacific region in the late 1980s (Boom, in Bates et al. 2010). Recently, Small et al. (2013) determined using ITS (internal transcribed spacer) sequences and PCR (polymerase chain reaction) analyses that the histophagous ciliate infecting the blue crab, *Callinectes sapidus*, in research facilities in Virginia and previously thought to be *Mesanophrys chesapeakensis* was actually *O. stellarum*. The same or similar infectious ciliate was probably responsible for histophagous disease in wild and captive blue crabs in Mississippi (Shields and Overstreet 2007), penaeid shrimps in Mississippi, and the wild lined shore crab, *Pachygrapsus crassipes*, from Carpinteria Salt Marsh, California, that we examined for Ryan F. Hechinger, University of California, Santa Barbara. This infection spreads in the partially closed circulatory system of the decapod (McGaw 2005) from the hemolymph to muscle, visceral organs, and other tissues (Figs. 2.3, 2.4, 2.5, and 2.6), usually killing the host. It has been assumed to enter into wounds of its hosts and now shown by Miller et al. (2013) to cause rapidly developing fatal infections in the blue crab inoculated with the ciliate or exposed to ciliates after experimental autonomy. When exposed to ciliates and not wounded, the crab seldom died. For comparisons, the fiddler crab *Uca minax* was inoculated with doses of either 10 or over 500 ciliates per crab, and crabs with 10 sometimes established infections, but those with the higher doses developed them rapidly. The infections developed at 10–15 °C, and ciliates were attracted to blue crab serum over other nutrient sources, suggesting the facultative nature of a blue crab parasite.

Viridans streptococci typically occur harmlessly in the mouth. These bacteria can be differentiated from *Streptococcus pneumoniae* using several tests.

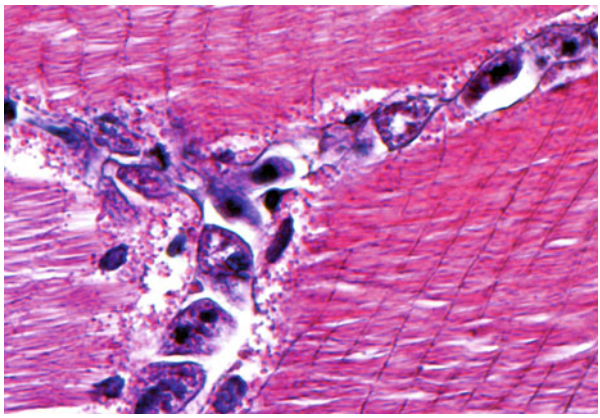

Fig. 2.3 The scuticociliate *Orchitophrya stellarum* or related ciliate in wild *Pachygrapsus crassipes* from Carpinteria Salt Marsh, California. Ciliates in hemolymph vessels beginning to erode adjacent skeletal myofibrils

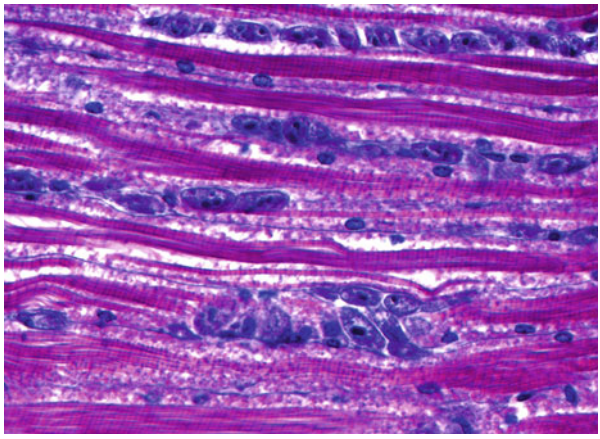

Fig. 2.4 *Orchitophrya stellarum* or related ciliate in *Pachygrapsus crassipes*. Heavily eroded muscle fibers

Moreover, they lack either the polysaccharide-based capsule typical of *S. pneumoniae* or the Lancefield antigens, based on the carbohydrate composition of bacterial antigens found on the cell walls in beta-hemolytic bacteria of the pyogenic, or pus-producing, members of the genus. Some may be involved in other mouth or gingival infections as pericoronitis or inflammation of the gums around molar teeth. However, if they are introduced into the bloodstream from surgical or other lesions, they have the potential of causing endocarditis, especially in individuals with damaged heart valves. Viridans streptococci have the unique ability to synthesize dextrans from glucose, allowing them to adhere to fibrin-platelet aggregates at damaged heart valves.

Often there occurs an indirect site-associated triggering mechanism for parasite establishment, with a corresponding associated ability to enhance or decrease pathogenic effects. The bothriocephalid *Anantrum tortum*, a long, up to over 15 cm, cestode, twists either singly or in groups of up to eight, within the intestine of its relatively small fish host, *Synodus foetens* (inshore lizardfish). It can occur

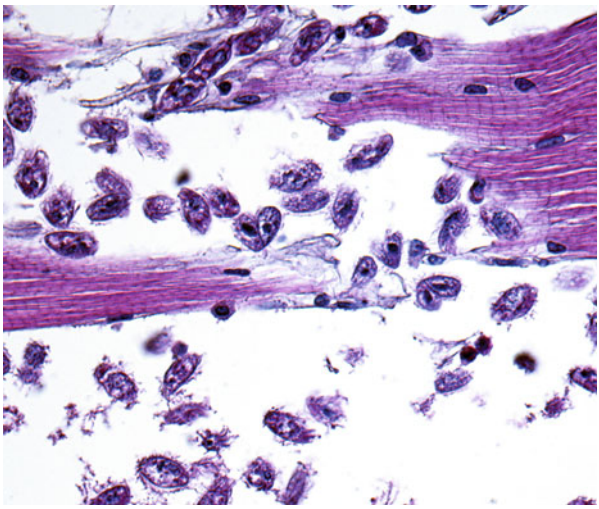

Fig. 2.5 *Orchitophrya stellarum* or related ciliate in *Pachygrapsus crassipes*. Severed muscle fiber showing clear view of ciliates

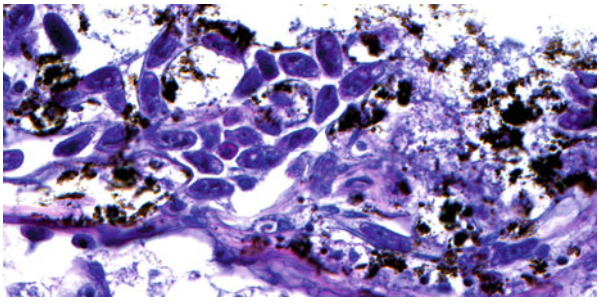

Fig. 2.6 *Orchitophrya stellarum* or related ciliate in *Pachygrapsus crassipes*. Infection showing associated melanistic response

near the pyloric ceca, along the intestine, or near the anus of the samples, measuring up to 300 mm long. Overstreet (1968) detected a 99 % level of significance over a 2-year period between the monthly prevalence of worms located near the anus and water temperature. Salinity was held constant, and an inverse sine transformation was applied to the percentage data. This anal location favors loss of the worm during warm weather, possibly assisting probability of best completing life cycle, but to be expelled, the lizardfish appears to have needed a several-mm-long packet of prey fish scales with a diameter much greater than that of the intestine. The carnivorous lizardfish can eat fishes and shrimps larger than itself. Consequently, fish prey with large scales tends to be advantageous for the lizardfish to rid itself of the parasite.

Sometimes, a fortuitous site creates a trigger for pathogenicity. For example, a diver accidently punctured his hand while reloading his spear gun. Later, unconcerned about his wound, he was filleting a jackfish, and a female of the nematode *Philometra* sp. invaded the wound and attached deeply. After several unsuccessful attempts to remove the worm, the pain-inflicting infection required surgery (Deardorff et al. 1986).

Not to be overlooked is the obvious matter of dose. This obvious matter is treated in discussions and is important for all agents from viruses to metazoans. In the case of metazoans, a host can come into contact with an especially high, unnatural dose of infective agents such as thousands of cercariae killing an intermediate host well before the corresponding metacercariae develop (e.g., Overstreet and Curran 2004). Intermediate hosts under specific conditions can be heavily infected simultaneously with several parasitic species. Some parasites such as microphallid trematodes infect a variety of birds and mammals. Therefore, a bird could acquire a harmful infection by feeding on individual heavily infected intermediate hosts, or source communities (Bush et al. 1993).

2.3.3 Host Species

The host species is also critical for many protozoans that are acquired by feeding on an intermediate host in which replication or maturation of a stage is necessary. For example, whereas a few killifish species are natural hosts of *Calyptospora funduli*, several related atheriniform fishes can be experimentally infected, but no nonatheriniform could be infected (Fournie and Overstreet 1993). Those experimentally infected demonstrated a variety of abnormalities, including asynchronous development, degeneration of early developmental stages, formation of macrophage aggregates, and a granulomatous inflammatory response, especially one exhibiting liver destruction in *Fundulus olivaceus* and *Rivulus marmoratus*. Of course, feeding behavior, environmental conditions, and geographic isolation can also serve as barriers to infection in fish in addition to innate immune barriers.

When introduced species become established in a system, they can be resistant to harmful factors in the environment, or they can be susceptible to extensive predation, diseases, and parasites. Species of the ascaridoid genus *Goezia* typically embed in an encapsulated ulcer in the wall as well as free in the lumen of the stomach of their natural fish hosts. These associations are described by Deardorff and Overstreet (1980a) for the nematode species *Goezia pelagia* in the cobia, *Rachycentron canadum*, and the Atlantic spadefish, *Chaetodipterus faber*, as well as for *Goezia minuta* in the gafftopsail catfish, *Bagre marinus*; the hardhead catfish, *Ariopsis felis*; and the inshore lizardfish, *Synodus foetens*. In none of these hosts does there appear to be any significant harm associated with the presence of the nematode (Deardorff and Overstreet 1980a). However, when the introduced host species such as the blue tilapia, *Oreochromis aureus*; striped bass, *Morone saxatilis*; and hybrid striped bass become infected with *Goezia sinamora*, the nematode produces massive fibrotic nodules in the fish stomach, and the nematode can also cause mortality. *Goezia sinamora* was implicated in mortality of hatchery-reared striped bass and tilapia introduced into a series of lakes in Florida, including Lake Parker [Gaines and Rogers 1972, see correction by Deardorff and Overstreet (1980a)]. In the tilapia, this roundworm has been observed to migrate through the intestinal wall, causing extensive lesions, in addition to forming nodules in the stomach. When the largemouth bass, *Micropterus salmoides*, and other native

species of fish were exposed to this nematode, the resulting infections contained relatively few of the roundworms and demonstrated no conspicuous harmful effect (Deardorff and Overstreet 1980a).

2.3.4 Host Modifications

Several books and chapters [such as those by Overstreet (1983), Barnard and Behnke (1990), Lewis et al. (2002), Moore (2002), Lefèvre et al. (2009), Adamo (2012), Adamo and Webster (2013)] have reported on parasite infections resulting in virulence, evolutionary, and behavioral changes in intermediate hosts such that the intermediate host has a better opportunity to be preyed upon by the appropriate definitive host than by chance alone. Whereas these changes are not necessarily triggers that make an agent more pathogenic, they often are changes that reflect the point at which the undeveloped, noninfective agent becomes infective to the definitive host and pathogenic to the intermediate host. A good example is the microphallid trematode *Levinseniella byrdi*, which infects the intestinal ceca of a few bird species such as the seaside sparrow, clapper rail, willet, and semipalmated sandpiper. These birds acquire the infection from one of a few talitrid amphipods such as *Uhlorchestia uhleri* from salt marshes of Texas to North Carolina and *Uhlorchestia spartinophilia* in a similar habitat from Cape Canaveral, Florida, to central Maine. In the Gulf of Mexico, the metacercaria also occurs in the amphipod species *Orchestia grillus* and *Chelorchestia forceps* (Bousfield and Heard 1986). The dramatic modification in the amphipod usually occurs after about a month, once the metacercaria becomes infective. At this point, the host amphipod turns from greenish or grayish to a translucent or bright orange (Fig. 2.7). The infected amphipod also unusually slows down its movements and, in contrast to its uninfected, negatively phototactic cohorts, does not always hide under wracks of dead, dissociated leaves and stems of spartina grass or other of their dietary debris shelters. Apparently, the amphipod carotenoids become unbound from protein of the infected host, allowing the bird host to be preferentially attracted to the now brightly colored amphipods containing infective metacercariae (Bousfield and Heard 1986). Johnson et al. (2009) experimentally manipulated a few tidal salt marsh creeks in Plum Island Estuary, Massachusetts, by nutrient fertilizer enrichment and exclusion of the killifish *Fundulus heteroclitus* (mummichog), a primary predator in the system. Interaction of the two treatments reduced abundance of the common *Uhlorchestia spartinophilia*, and apparently infected amphipods moved from the marsh edge to the adjoining creek-wall habitats during 1 year, resulting in 97 % of the amphipods clinging on the creek walls and exhibiting the bright orange. A subsample of the colored amphipods was confirmed to be infected, and they were seen being fed upon by the semipalmated sandpiper and seaside sparrow.

In regard to the above host modification, other microphallid species in those amphipods did not turn their hosts orange or noticeably modify their behavior. A similar situation has been noted to occur where *Levinseniella tasmaniae* but not

Fig. 2.7 Specimens of the amphipod *Orchestia grillus* in coastal Mississippi showing on the *top* an uninfected one and on the *bottom* a transformed orangish one that is infected with the microphallid *Levinseniella byrdi*, a trematode that infects the intestinal ceca of a few bird species. The transformation triggers phenotypic and behavioral changes specifically attracting infective specimens to predatory birds in which the trematode matures

other microphallids induced an orangish color in the amphipod *Austrochiltonia australis* in Tasmania (Smith 1981). That is not to say all species of *Levinseniella* induce coloration and other modifications in their amphipod hosts since *Levinseniella tridigitata* does not modify *Gammarus aequicauda* (see Thomas et al. 1996) and *Levinseniella carteretensis* (or *Levinseniella hunteri*) does not induce a color change in any of the five talitrids, including *U. uhleri*, when experimentally infected (Bousfield and Heard 1986, Heard personal communication). Another trigger involves photoreception. The snail host typically sheds large quantities of cercariae of *Levinseniella tasmaniae* infective for the amphipod host when under light conditions, whether natural or experimentally reversed (Smith 1981). And yet, cercariae of other species are shed during dark or other physical conditions.

2.3.5 Combination of Many Factors

Almost all symbiotic relationships, including those discussed above, involve a combination of triggering factors. Those factors and host–symbiont relationships involving termites are particularly well studied. We, ourselves, have examined in

considerable detail the host–symbiont relationships affecting the outcome of pathogenic viruses in populations of commercial penaeid shrimp. We will address in this section some of the symbiotic relationships and interrelationships that can involve a shift from harmless to harmful relative to three host groups.

2.3.5.1 Termites

The relationships between termites or related insects and their symbionts are numerous and provide examples of an abundance of triggers, including diet, to control the various relationships. Some of these involving termites will be discussed elsewhere by David Bignell (Volume 2, Chapter 6 of this series). Investigations by L. R. Cleveland (e.g., 1926) were designed to better understand symbiosis using termites and their intestinal flagellates. He was well aware that bacteria and yeasts were involved in the relationships and that there was a fine line separating where one symbiotic association ended and the other began. For example, when one agent in a so-called mutualistic relationship could survive without the host, it nevertheless also can become a parasite living off the host. This situation, however, is complicated and difficult to assess. He tried to remove one or more flagellate microorganisms from a host without harm to the host so that the association could be manipulated. He also thought it best to consider all components, which he defined differently than we do (he considered a commensal association to be one when neither party was benefited nor injured), of an association as symbionts. Depending on a specific termite host, he could void the flagellates with starvation, a high temperature not lethal to the host, a specific level of moisture, and oxygen under pressure. For example, by oxygenating the large Pacific Coast termite (*Zootermopsis nevadensis*) for 7 h at 1.5 atm, two (*Leidyopsis sphaerica* and *Trichonympha campanula*) of the four flagellates survived, and the host lived "indefinitely." Additionally, starving the termite at the above conditions for 6 days left only one flagellate (*L. sphaerica*), showing that both *L. sphaerica* and the termite do together constitute a necessary and mutualistically beneficial symbiotic relationship. Without any flagellates but with a diet of wood, the termite lived for about 3 weeks. Reintroducing either *L. sphaerica* or *T. campanula* allowed the termite to experience its normal much longer longevity (usually 60–70 days). Cleveland realized that the presence of the other two flagellates (*Trichomonas termopsidis* and *Streblomastix strix*, both having a poorly understood symbiosis with epibiotic bacteria) in the termite starved for 8 days would help the termite survive for about 10 weeks but were not necessary symbionts; he realized that bacteria played a role in digesting the diet. The "primitive" termite *Mastotermes darwiniensis* from Australia was studied by Li et al. (2003), and it had in its hindgut six flagellates, none of which can yet be cultured. Historically, these flagellates were considered to use their digestive enzymes to digest cellulose for the benefit of the termite. However, they determined using PCR technology that the main endoglucanase activity in the flagellates appears to originate from termite cellulases produced in the salivary glands. At least two of the flagellates possess their own

endoglucanase genes, which are expressed but without significant enzyme activity in their nutritive vacuole. After millions of years of evolution, these flagellates, suggested by Li et al. (2003), are heading for a secondary loss of their own endoglucanases to exclusive use of the termite cellulases. Feeding on the symbionts still seems to be an important nutritional component of the termite diet.

Some termites, such as those which are soil-feeders, depend entirely on bacterial symbionts (Bignell et al. 1980). This association, often involved with coprophagy, feeding on fecal pellets containing termite bacteria that digest the cellulose, will be treated by D. E. Bignell elsewhere in this series (Volume 2, Chapter 6). Other termites, such as members of the *Macrotermitinae*, depend on a relationship with mutualistic fungal symbionts of the genus *Termitomyces*, which form fungal combs in the nests. These and attine ants can produce nests that often are thousands of liters in volume, able to persist for decades, and contain millions of sterile helper individuals usually resulting as offspring from a single queen. Those termites are major decomposers of the Old World tropics, and the ants are dominant herbivores of the New World tropics. The fungal symbionts of the termites can produce sexual fruiting bodies allowing their horizontal acquisition, but those of the attine ants rarely fruit and are typically propagated clonally and vertically by the dispersing queens. The life cycles of the fungus are again shown to help trigger different symbiotic relationships. Aanen et al. (2002) present phylogenies of the termites (about 330 species in 11 genera) and their fungal symbionts which number about 40 and are shared by different termite species. They show significant congruence between the termite and fungal phylogenies because the interactions at higher taxonomic levels show considerable specificity. They also considered the trait of biparental colony founding to constrain evolution of vertical symbiont transmission in termites, where the male survives and mates repeatedly with the female for life but not in ants where males die after a single mating. The Formosan subterranean termite pest (*Coptotermes formosanus*) builds a large nest with spongelike networks of intricate feces-lined tunnels (carton material). Fungal pathogenic agents are used, usually unsuccessfully, to kill the termite. Studies by Chouvenc et al. (2013) have shown that environmental conditions within termite nests promote the growth of Actinobacteria, whose presence in turn seems to protect the termite colony against fungal entomopathogens, including *Metarhizium anisopliae*. In other words, the Actinobacteria, which represented a nonnutritional exosymbiosis in the termite, was a defensive mutualism that increased survival of the termite and was additive to the termite's individual immunity and social defensive capacity, which in turn increased survival of the termite.

2.3.5.2 An Endemic Virus

Baculovirus penaei, commonly called BP or BP (PvSNPV), has a widespread distribution in cultured and wild penaeids, and it can cause severe epizootics in larval, post-larval, and juvenile stages. It is an enveloped, polyhedrosis, rod-shaped, double-stranded, intranuclear, DNA virus infecting epithelial cells of either the

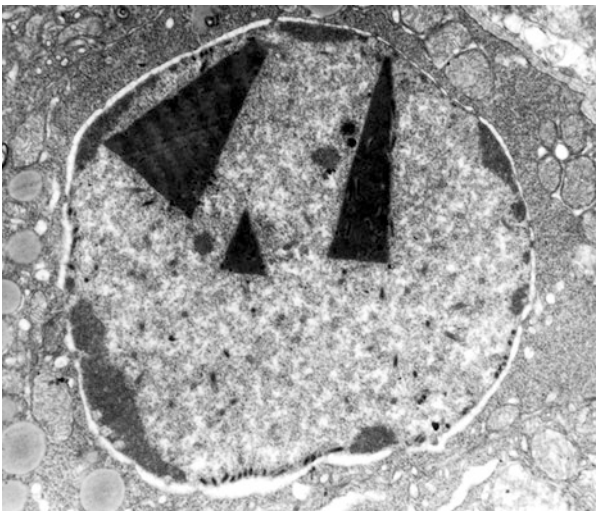

Fig. 2.8 About 2–3 day infection of *Baculovirus penaei* (BP) infecting hepatopancreatic tubules (HP) of larvae of *Litopenaeus vannamei* demonstrating triggering condition for viral replication and resulting pathogenic features in larval and young post-larval individuals that are rare or absent in juvenile and adult shrimp. Ultrastructure of hypertrophied nucleus of infected cell showing emarginated chromatin, developed virions lining internal surface of the nuclear membrane, replicating developing nucleocapsids in nucleoplasm, and polyhedra incorporating virions. Note abnormal mitochondria in cytoplasm surrounding nucleus

anterior midgut and midgut gland, or the hepatopancreas (HP; R-cells, F-cells, and B-cells, most commonly found near the base where the HP tubules join the anterior midgut). The E-cell populations (stem cells) at the distal portion of the hepatopancreatic tubules produce cells so quickly as to assure noninfected new cells (Figs. 2.8, 2.9, and 2.10) (Overstreet et al. 1988). Baculoviral infections of BP can involve nearly 90 % of the shrimp HP during mysis and early postlarvae stages without necessarily causing death. A number of triggering factors can tip these acutely infected shrimp into mortality. The virus is one out of 25 or so that infects penaeid shrimp and one of a few thousand known from invertebrates. Because it has a proteinaceous tetrahedral occlusion body, infections can be detected and followed with a light microscope; because of these bodies, it was the first species reported and characterized from a shrimp (Summers 1977).

The virus has a simple direct life cycle, although unknown means allow the agent to remain dormant or in a reservoir host. It can infect an acceptable penaeid either from free or occluded virions (Fig. 2.8) in the surrounding water or through a carrier host. Experimental infections are best accomplished by feeding the virus concentrated in a rotifer to protozoeal or early-stage mysis larvae or concentrated in a brine shrimp to infect late-stage mysis or early postlarvae (Overstreet et al. 1988). Depending on a number of factors, the virus replicates in the host alimentary cells and is most prevalent and infective at about day 3 after being fed. The nucleus of those cells enlarges, and the polyhedra and associated virions rupture into the lumen

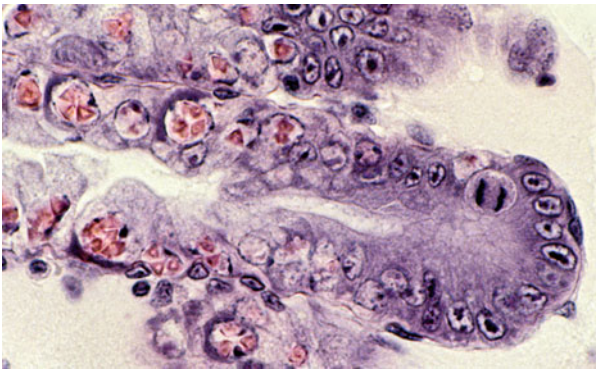

Fig. 2.9 *Baculovirus penaei* in HP of 2–3 day old larva of *Litopenaeus vannamei*. Histological section (stained with hematoxylin and eosin) showing tip of HP tubule with few actively dividing embryonic cells (E-cell, note large mitotic nucleus) with distal to proximal cells showing a progression of enlarged nuclei and large polyhedra

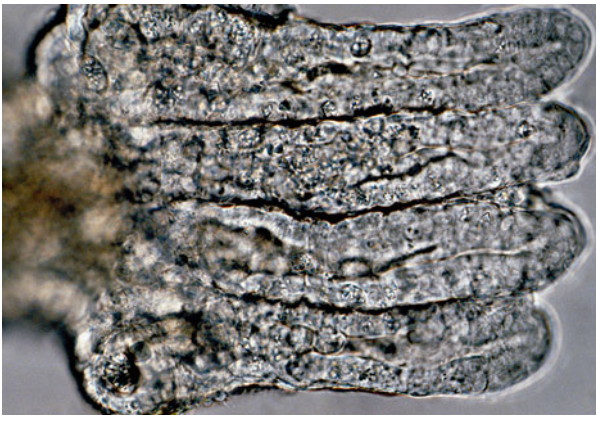

Fig. 2.10 *Baculovirus penaei* in HP of 2–3 day old larva of *Litopenaeus vannamei*. Fresh HP tubules showing small- to medium-sized polyhedra infecting most cells other than those at the tips

of the host midgut. We have observed as many as 24 relatively large tetrahedral bodies (about 13 μm on a side) or 100 small distinct bodies in experimental infections. The infective agent is then passed out through the intestine or it can be released when the infected larva is eaten by an appropriate predator. Unlike some insect baculoviruses that are used to kill agricultural pests, the BP virion remains active in the polyhedron for less than 2 weeks rather than for 80 or so years. On the other hand, BP maintained in an ultralow freezer remained active for at least three and a half years.

Natural infections of BP in the brown shrimp, *Farfantepenaeus aztecus*, were monitored monthly between January 1989 and November 2000 with seasonal

samples conducted until May 2004. The monitoring took place in the northern Gulf of Mexico in or off Mississippi waters, but shrimp from Florida were also examined. During the mid-1970s, infections occurred in wild populations of the pink shrimp, *Farfantepenaeus duorarum*, but when we monitored all commercial penaeid shrimps for those infections, the virus primarily infected the brown shrimp and only occasionally infected the pink or white shrimp, *Litopenaeus setiferus*, which exhibited the otherwise rare infection. During the monitoring, we tried to use 4-cm long shrimp because they exhibited the highest prevalence and intensity of infection. Infection usually occurred only about 3 or 4 months of the year between about April and June, with the highest prevalence occurring in 1998 when we detected values of 56, 10, and 4 %. Values reaching 40 and 36 % occurred in May 1993 and 1997, respectively. Typically, the prevalence reached about 10–25 % during the remaining years, and infections were almost always absent in fall and winter. Rarely was the intensity of infection great, as usually observed in experimental infections with larvae and early postlarvae. The outbreak dynamics probably resulted from a variety of factors, including genetics (primarily brown shrimp), the interaction between the life cycle of the shrimp and the presence of the virus, the age of shrimp when initially infected, and resistance to infection, which occurred primarily in larger shrimp. These factors will be discussed below in more detail such as with infections and problems in aquaculture hatcheries.

Viral Factors

Strains There are at least four strains of BP as differentiated with molecular probes. Each seems to be restricted to specific penaeid shrimps. One strain occurs in some commercially important shrimp in the northern Gulf of Mexico; another occurs in noncommercially important shrimp (*Trachypenaeus* spp.) in the northern Gulf of Mexico; another occurs in *Melicertus marginatus* in Hawaii; and the last occurs in *Litopenaeus vannamei* in Ecuador. We have used for our experimental research the latter strain originally obtained and frozen from Ecuadorian aquaculture facilities by biologist James Brock. There is also a series of strains of MBV (*monodon* baculovirus), a baculovirus that produces spherical polyhedral bodies but which have many similarities to BP. We attempted cross infections with BP for all but the Hawaiian strain, using presumably susceptible penaeid postlarvae without success.

Free and occluded virions In addition to the virions occurring in the tetrahedron, which occasionally does not contain any virions, free ones lined up along the abnormally-distorted internal surface of the nuclear membrane. The free viral particles were collected from a freeze-thaw preparation either by filtration through a 0.45 μm filter or from processing of the preparation by relatively low-speed centrifugation to prepare a cell-free supernatant, and when next exposed to shrimp larvae, they produced infections. These subsequent infections did not appear to be more virulent than those initiated from virions incorporated in the tetrahedron.

However, the free virions of some insect baculoviruses produce secondary infections in the hemocoel, rather than gut cells, and those are considerably more virulent (Kelly 1982).

Dose Dose of BP is difficult to determine because there is no appropriate crustacean cell line available to culture the virus. However, we conducted a replicated relative study using the same standard viral stock suspension and determined a clear dose response involving both prevalence of BP and mortality of shrimp (Overstreet 1994).

Environmental factors Temperature and probably other factors affect both the production of free virus and the relationship between the virus and its shrimp host. When a homogenate of BP-infected cells is placed in seawater (32 ppt), the virus becomes completely inactivated between 7 and 14 days when maintained at 22 °C. However, when maintained at 5 °C, some virions remained active after 14 days. A low temperature also delayed or inhibited infections in larvae and presumably adults. Virions were inactivated by a 10-min exposure to temperatures of 60–90 °C.

A variety, but not all, of toxicants can probably affect the relationship between the viral agent and shrimp host. Couch (1976) reported an increase in prevalence of infection in pink shrimp exposed to low levels of Aroclor 1254®. We tried to duplicate his experiments using brown shrimp and 3 ppb Aroclor 1254 and also 2 ppm nickel but with unsuccessful outcomes. We later discovered from Couch (personal communication) that the Aroclor 1254 he used was probably unknowingly contaminated with another toxin (Overstreet 1994).

Studies were conducted by treating free BP in a variety of ways and then feeding it to larvae of *L. vannamei* as a bioassay (Overstreet et al. 1988). Unlike insect baculoviruses, all of the BP virions that we tested were inactivated when desiccated for 48 h. Tests conducted for possible treatments in aquaculture also showed that the virus was completely inactivated by a 30-min exposure to pH 3; pH 11 extended the pre-patency period of infection, but it did not inactivate the virus. Ultraviolet irradiation for 40 min at a wavelength of 254 nm when the virus was 5 cm from the light source also inactivated the virus (LeBlanc and Overstreet 1991a). The virus was also completely inactivated by a chlorine (in the form of calcium hypochlorite) concentration of 200 mg/L when treated for 1 h and by 1600 mg/L when treated for a time period as short as 20 s (LeBlanc and Overstreet 1991b). Other than for sterilizing aquariums, these methods other than desiccation and steam cleaning seemed impractical for an aquaculture facility.

Host Factors

Research host shrimp To conduct experimental infections, we used mostly *Litopenaeus vannamei* cultured at the Gulf Coast Research Laboratory Consortium either at Oceanic Institute (OI) in Hawaii or at GCRL in Ocean Springs. Our early

studies with larvae required antibiotics to counter bacterial infections, which otherwise often reduced a larval stock by 50 % and would have been considered an acceptable loss in commercial hatcheries at that time. Even with antibiotics, we obtained BP infections with associated catastrophic mortalities when the shrimp larvae were exposed to the virus. After hybridization crosses of shrimp and introducing special wild brood stock, the result was creation of shrimp stocks with improved resistance to BP, and we noted as a bonus our having created crosses exhibiting resistance to other microbial agents. The BP prevalence could be variable, but mortality was reduced or eliminated. In a few cases, we obtained shrimp from commercial hatcheries or other research facilities and had to use antibiotics.

Host resistance The genetic families of *Litopenaeus vannamei* produced at OI gradually became more resistant to BP. In some cases, it was easy to obtain 100 % prevalence but without the associated mortalities observed using other stocks of shrimp. Middlebrooks et al. (1989) examined lectin levels in the hemolymph of monthly samples of wild *Farfantepenaeus aztecus*, and the levels were found to vary by season and among individuals, suggesting that levels could serve as indicators of health or immune status. High titers of activity for lectin agglutination occurred in September, and low levels occurred in April. Those observations of positive BP infection results when lectin levels were low corresponded with the time when BP first became apparent in the wild shrimp population, and polyhedra subsequently were no longer apparent when lectin levels were high.

Age and growth The age of *Litopenaeus vannamei* influences BP infections in a variety of ways. Stuck and Overstreet (1994) exposed pathogen-free shrimp ages mysis 2–3 through 25-day-old postlarvae (PL) from different sources, each with a single viral exposure, and cultured them for 15–21 days. All groups PL9 or younger became heavily infected within 2–5 days, some experiencing high mortalities compared with controls. Surviving postlarvae had reduced growth as determined by dry weight. One of these groups was cultured for an additional 49 days, and the smaller postlarvae that survived the infection appeared similar to the controls after 4 weeks by which time viral prevalence had decreased. Exposure of the virus to older postlarvae produced a high prevalence of infection but with little effect on either survival or growth. When 13- to 14-day-old postlarvae in similar size groups of previously infected and noninfected individuals were starved for 10 days, less than 2 % of the infected postlarvae survived the 10-day period compared with 52 % of the noninfected ones. Leblanc and Overstreet (1990) exposed infected BP to groups of *L. vannamei* 3, 39, 63, 120, 157, 325, and 454 days after reaching postlarvae. The postlarvae exposed when 3-days-old (PL3) exhibited infections in 77 % of those examined at 5 days postinfection (PI) and in 100 % of the same group when examined at day 9 compared with 0 % prevalence in the controls; 14 % of the shrimp that were exposed when postlarvae for 39 days (PL39) had developed an infection at day 9 PI when 20 % of those exposed as PL63s exhibited infections; and the prevalence in the latter group increased to 42 % at day 14. With the exception of one shrimp exposed when a PL120, none developed an observable infection at 19 days, and only 2 of 20 of the exposed PL157 group examined at day 16 exhibited

an infection. No control became infected. Other experiments also showed this decrease in prevalence as well as intensity of infection and mortality with age. In general, most postlarvae 12 days old or older did not become infected, and those that became infected did not die. This age corresponds with the age that most postlarvae leave the offshore waters and settle on the substratum in the estuarine area where the infective agents occur.

Energy The nutritional condition of *Litopenaeus vannamei* can be assessed biochemically in terms of available energy reserve. The principal energy storage materials in the penaeid shrimp are lipids and proteins, with carbohydrates considered to be a minor energy reserve. Triacylglycerol (TAG), an ester derived from glycerol, and three fatty acids serve as the primary classes of lipid used for energy storage. In patent infections of BP conducted by Stuck et al. (1996), the polyhedra first appeared in the HP 18–24-h postexposure with a maximal infection usually occurring at 72 h (also see Hammer et al. 1998); however, progression of the infection after the initial appearance of the polyhedra was variable among shrimp potentially corresponding with different levels of body lipid used for energy storage. We measured preinfection and postinfection TAG and protein levels during a series of experimental BP exposures using shrimp from a variety of sources with different inherent protein and TAG levels. Mysis-stage or early post-larval protein levels measured either preexposure or postexposure to BP showed no consistent relationship with BP infections. However, a viral prevalence of 86–100 % occurred at 72 h when initial TAG levels measured above 3.5 μg/mg in contrast to 35 % or less in shrimp when initial TAG levels <2.0 μg/mg. TAG levels in mysis stage-1 larva rose rapidly from 1 to about 11 μg/mg in 2 days for both infected and negative control populations, then dipped to about 2 μg/mg at day 9, and subsequently again rose in both populations but was about 3 μg/mg higher in the infected shrimp at day 15. Most shrimp died before the end of a week. Two other experiments starting with 9-day postlarvae (PL9s) were conducted when initial TAG levels were either about 1 or 7 μg/mg, and substantial mortalities did not occur in either. All individuals in the infected groups became infected, at about day 10 in the initial low TAG group and at day 3 in the initial high group. In the initial low TAG group, the TAG level of the infected group stayed about the same at day 20, but that of the control groups increased to about 4 μg/mg. In contrast, in the initial high TAG experiment in which the TAG level for both infected and control groups dipped from 7 to about 1.5 μg/mg at day 3, the level increased to about 10 μg/mg at day 20 and about 15 μg/mg at day 25. Stress caused by starving postlarvae (PL18s) reduced TAG but not protein reserves. Detection of the BP infection was delayed for 30 h when the group to be infected was starved for 48 h compared with continuously fed controls, but the prevalence of infection in both groups increased rapidly to similar high prevalence values above 80 % between 72 and 192 h PI.

Genetics Offspring of male and female crosses of high-growth and low-growth "families" of a well-defined population at GCRL Consortium at OI, Hawaii, were fed BP as 15-day-old postlarvae (PL15s) (Alcivar-Warren et al. 1997). The

high × high-growth and low × high-growth (female × male) postlarvae, respectively, had a 77 and 85 % survival rate at 18 days postexposure compared with 19 and 24 % survival for the low × low-growth and high × low-growth offspring. All but the high × low-growth offspring, with a 68 % prevalence of infection, had an 88–100 % prevalence at day 4. The low × low-growth cross of 3-month-old shrimp fed the virus IHHNV (infectious hypodermal and hematopoietic necrosis virus, which has been classified as *Penaeus stylirostris* densovirus) exhibited the highest prevalence (48 %) at 30 days compared with the lowest (6 %) in the high × low-growth cross. Random amplified polymorphic DNA polymorphisms for the four crosses showed no clear relationship between the prevalence values of IHHNV and BP. Even with similar mtDNA haplotypes included in the initial crosses, the offspring of those crosses exhibited major differences in both steady-state levels and patterns of expression of mitochondrial 12s rRNA at various early developmental stages of the resulting offspring of the different crosses. Even though specific genetic markers and differences could not be associated with specific differences in susceptibility to different infections, shrimp growth, or regulation of gene expression, the genetics of the shrimp play an important role in triggering infections and mortality from infections.

Concurrent agents influencing infections Reo-like viruses in crustaceans often occur concurrently with other disease-causing viruses producing a synergistic effect. We (Krol et al. 1990) found such a virus infecting the anterior midgut epithelium and the R- and F-cells from the HP in larval specimens of *Litopenaeus vannamei*. The occurrence of the reo-like virus was observed only in BP-infected shrimp; however, shrimp with BP seldom had the reo-like virus. Other agents often associated with stress also occur in shrimp (Overstreet 1994).

Susceptibility to viral infections appears to be enhanced by crowding of host individuals. We determined that the prevalence and intensity of infections in shrimp crowded in commercial bait tanks were higher than in those collected from the wild shrimp population that was used to stock the bait tanks.

In summary for the endemic virus BP, there are a variety of susceptibility factors dealing with the environment, virus, and host, including the nutritional and molting state of the host, which can trigger a relatively harmless infection to develop into a severely pathogenic condition. In nature, this condition is seldom recognized because only microscopic-sized larvae and early postlarvae usually die from the infection, dead animals cannot be seen because they are small or readily eaten, and the opportunity for the agent and the larvae to come into close proximity is relatively rare. In aquaculture, the virus and larvae can and do come into contact. Hatcheries in the multibillion-dollar shrimp industry have prompted research to solve the previous major threat that collapsed hatcheries in the USA and elsewhere in the Western Hemisphere during the early days of the industry when brood stock was obtained from the wild. The related MBV in the Eastern Hemisphere also caused a similar major problem. Now that the industry can rear most penaeid species; detect BP and other infectious agents with PCR, gene probes, and other methods in routine monitoring; produce its own brood stock relative to disease

resistance and growth potential; and accommodate ambient temperatures, infections of BP in aquaculture are rare. If they do occur, an entire spawn can be destroyed and disposed and the system decontaminated, and there would be a time loss of only a few days before a new spawn could be produced.

2.3.5.3 Exotic, or Introduced, Viruses: The Emergence of Viral Pathogens of Shrimp Aquaculture

With the exception of baculovirus species, most or all the pathogenic penaeid viruses are not native to the shrimp species being cultured or perhaps to any commercial shrimp. Consequently, the cultured species may be highly susceptible to and die from one or more of the viral agents. Most of the presently known 25 or so shrimp viruses cause mortality in subadult animals that have been reared for a few months. The high cost of feed and labor plus the relatively long animal growing period results in a high economic cost. Therefore, it is necessary to incorporate the costs of an inability to quickly replace the entire infected pond-reared adult or subadult stock as can be done for larvae in hatcheries infected with BP infections. Production losses associated with the exotic viruses white spot syndrome virus (WSSV) and Taura syndrome virus (TSV) provide excellent examples for examining the emergence of pathogenicity in aquaculture on a large scale.

The history of the shrimp aquaculture industry is a case study of the emergence of severe pathogens in hosts where none was known previously. For about two decades after the industry began in the 1970s, few viral pathogens were known. Beginning in the early 1990s, several severely pathogenic viral diseases emerged. We will consider two of the pathogens in some detail as they inform our understanding of the appearance of highly pathogenic symbionts through host switching.

White spot syndrome virus (WSSV) was first reported in 1992 from Taiwan. The virus belongs to the order Baculovirales. Although once considered a member of the Nudiviridae or Baculoviridae, WSSV is more closely related to the recently erected family Hytrosaviridae (Wu et al. 2009; Wang and Jehle 2009). WSSV is now considered a member of the genus *Whispovirus* in the family Nimaviridae. The virus is a rod-shaped, enveloped virion and among the largest viruses known at 120–150-nm wide by 270–290-nm long. The genome is double-stranded DNA of 295 kbp. The host range is among the widest of crustacean viruses and infects all decapod crustaceans that have been tested.

Taura syndrome virus (TSV) was first observed from Ecuador also in 1992. The virus belongs to the order Picornavirales and is a member of the family Dicistroviridae and the genus *Aparavirus*. The virion is icosahedral, nonenveloped, and 32 nm in diameter. The genome contains positive sense, single-stranded RNA of 10 kb. The host range is restricted mostly to members of the genus *Penaeus* sensu lato, although recently Overstreet et al. (2009) reported experimental infections with replication in at least some symbiotic barnacles. Pathogenicity varies with host species being most virulent in *Litopenaeus vannamei* and less so in other species of penaeid shrimp (Overstreet et al. 1997).

Although it is possible that the viruses were symbionts of wild shrimp prior to being detected in shrimp aquaculture, we wish to explore the hypothesis that they emerged by host transfer from insects or other terrestrial arthropods during the development and expansion of shrimp aquaculture. Roekring et al. (2002) perhaps first suggested this tantalizing hypothesis in their study of three shrimp parvoviruses whose closest known relatives are parasites of insects. Host switching is a common mechanism for the emergence of pathogenic pathogens. For example, in humans, 75 % of today's emerging human viral diseases are zoonotic, e.g., HIV-1 and HIV-2, influenza virus, Ebola virus, hantaviruses, Nipah virus, Zika virus, and the SARS coronavirus that causes severe acute respiratory syndrome, and many of the rest of human infectious diseases have their closest relatives residing in nonhuman animal hosts (Parrish et al. 2008; Pearce-Duvet 2006).

Most theories of the emergence of new diseases through host switching recognize several steps: cross-species transmission, establishment, and spread (Parrish et al. 2008; Domingo 2010; Antia et al. 2003). The first step in the process is an increase in the number of contact events between the recipient host and the donor host. The increase in contacts is usually due to an ecological change or disturbance. As the number of contacts between the donor and recipient hosts increases, there may be a concomitant increase in successful transfer of the pathogen. In the case of shrimp viruses, we suggest below that there was an increase in contact between penaeid shrimp and other arthropods during the early 1990s, and that facilitated cross-species transmission and the emergence of pathogens in penaeids.

Although cross-species transmission may occur, permanent establishment within the new host requires enough within-species transmission to maintain the pathogen in the new host. It is unknown how many times the ancestor of these two symbionts was transmitted to penaeid shrimp only to be eliminated because shrimp-to-shrimp transmission was not sufficient. However, at some point, either prior to cross-species transmission or after initial colonization, a mutation of the ancestor resulted in penaeid shrimp being highly susceptible to them, resulting in the maintenance of the infection locally in shrimp aquaculture.

A third set of factors allows for the pandemic spread. Sharp and Hahn (2010) review the successful establishments of HIV from primates to humans. Although there may have been as many as seven successful transmissions, most are restricted to Africa, and only one of the seven establishments of HIV (HIV-1-M) is responsible for the pandemic of HIV in the 1980s (Sharp and Hahn 2010; Pepin 2011). One or more factors other than trans-species transmission allowed for the amplification and spread after a transmission. In the case of HIV, it is thought that the increased use of vaccination in colonial Africa, chance, and increased world travel may have been the contributing factors (Pepin 2011). In the case of shrimp viruses, it may be that the greater number and higher shrimp densities in aquaculture settings coupled with the long-distance transport of live and fresh frozen shrimp has allowed worldwide viral spread after cross-species transmission.

The rapid expansion of the shrimp aquaculture industry in the 1980s and 1990s brought with it conditions conducive to increasing the rate of contact between shrimp and terrestrial arthropods, the majority of which are insects. According to

FAO statistics (FAO 2009a, b) in the late 1970s, most of the penaeid shrimp were coming from capture fisheries (Fig. 2.11). However, between 1992 and 1993 production of penaeid shrimp from aquaculture surpassed 50 % of all penaeid shrimp production worldwide (Fig. 2.12). This local peak occurred near the time that the two viruses appeared in shrimp aquaculture. Subsequently, culture production declined as a percentage of total penaeid production and remained below 50 % throughout the 1990s. However, the first decade of the twenty-first century saw a substantial increase and by 2010 the contribution from aquaculture to world's commercial shrimp increased supply by nearly 75 %.

Clearly, in the early 1990s, when viral pathogens emerged, there were twice as many penaeid shrimp in the market place as there had been a decade before. Although this does not mean that the total number of shrimp in the world doubled (not all of the shrimp in the world were captured), it does suggest that aquaculture substantially increased the total number of penaeid shrimp in the world and continues to do so. It is also clear that the increased number of aquacultured shrimp were at higher densities than wild shrimp. This increased population size and density are conducive to the spread of infectious diseases.

Perhaps more important for the cross-species transmission of the viruses from insect to shrimp is the habitat of those new shrimp. For the most part, they were in earthen ponds in the coastal zone. However, many of the ponds were located a distance from the coastline, and seawater was pumped over a considerable distance to provide the necessary culture medium. The point is that there may be more shrimp that were likely to have contacted terrestrial insects and other terrestrial arthropods, after the expansion of shrimp aquaculture than before it.

The second point in building a case for the origin of the two shrimp viruses from insects or other terrestrial arthropods is that the hosts of the viruses most closely related to shrimp viruses are insect or terrestrial arthropod hosts. Roekring

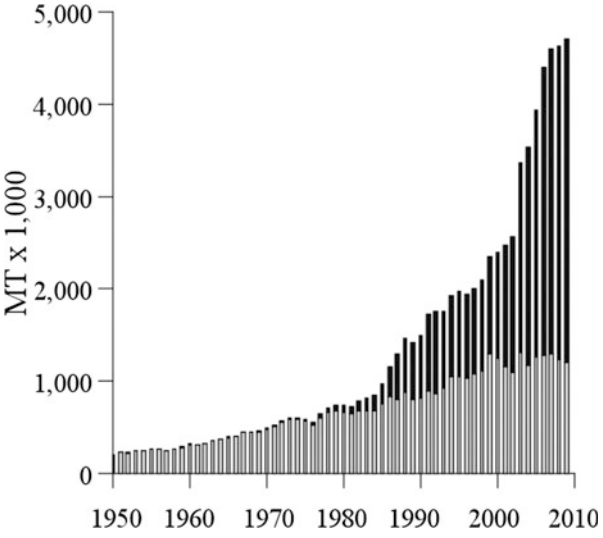

Fig. 2.11 Total world's supply of penaeid shrimp in metric tonnes. Production from the capture fishery is *light bars*. Production from aquaculture is in *dark bars*. Source FAO statistics (FAO 2009a, b)

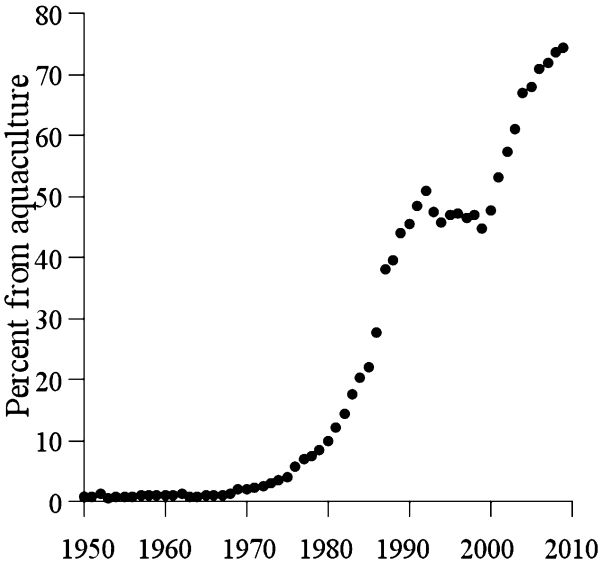

Fig. 2.12 Proportion of world's penaeid shrimp from aquaculture. Note that the proportion of penaeid shrimp production saw a local peak in the early 1990s above 0.5 and then declined during the emergence of Taura syndrome virus (TSV) and white spot syndrome virus (WSSV). Source FAO statistics (FAO 2009a, b)

et al. (2002) indicate that shrimp parvoviruses do not form a single clade but are distributed among clades that include insect parvoviruses. Roekring et al. (2002) suggested that viral transfers between crustaceans and insects occurred.

Most members of the Dicistroviridae are found in insects (Baker and Schroeder 2008). Guo et al. (2013) noted that the exceptions are TSV and the recently-described *mud crab dicistrovirus-1* (MCDV-1). MCDV-1 groups with TSV which could indicate that TSV in shrimp aquaculture arose from other crustaceans. It should be noted that the first reports of TSV were from the New World in the 1990s and that MCDV-1 was described from Asia long after TSV was transferred from the New World into Asia in 2000, suggesting that MCDV-1 arose after TSV was introduced into Asia.

Similarly, WSSV as a member of the order Baculovirales has as its closest relatives symbionts of insects. In particular, Jehle et al. (2013) group WSSV most closely with two insect viruses causing salivary gland hypertrophy, *Musca domestica salivary gland hypertrophy virus* (MdSGHV) and *Glossina pallidipes salivary gland hypertrophy virus* (GpSGHV).

We have provided a number of examples and triggers of the switch from one quality of a symbiotic relationship to another, specifically from commensal to pathogen. Examples of triggers include environmental ones like temperature, toxic chemicals (dose), chemotherapeutics, dietary changes, and geographic habits; internal ones include host physical site, host resistance or susceptibility, host modifications, and host switching; and combinations of these and other conditions.

2.3.6 *Evolutionary Context for Changing Pathogenicity*

The question arises, "Is becoming a pathogen an adaptation for a symbiont?" In an evolutionary context, which is where the concept of adaptation lies, why would a symbiont switch to being pathogenic, either at some stage in its life cycle or in some hosts at the same stage but not in others?

In some cases, symbionts are pathogenic as a clear adaptation, e.g., the *Levinseniella byrdi*, referenced above causes damage or even death of the intermediate host to complete its life cycle. In particular, *L. byrdi* causes a phenotypic change in the host that increases the host's chances of becoming a prey. In other cases, it is not so clear. Facultative symbionts, such as entomophagous nematodes, are both free living and symbiotic (Sudhaus 2008). The adaptation of being symbiotic is that it increases the habitat breadth of the symbiont, and inhabitation of a living organism removes the organism from competition with other organisms encountered living freely in the habitat. Thereby, the relationship allows the symbiont an escape from competition. So that may be the reason for a life history strategy in which both symbiotic and free-living phases exist. However, we are most interested in answering the question of why would a symbiont become pathogenic in some hosts and not in others from an evolutionary perspective.

There is a body of theory that addresses virulence evolution in symbiotic organisms. Traditionally, the virulence of a pathogen was thought to be a reflection of a recent host–symbiont association and that over time there would result a diminishment in virulence of the pathogen (May and Anderson 1983; Anderson and May 1992; Ewald 1994). It was argued that no symbiont would benefit from killing its host (destroying its habitat and thereby destroying itself). However, this thinking fails to recognize that natural selection maximizes reproductive success of the organism. Reproductive success is a composite of births (new infections) and survival (loss of infection) not just survival.

More recent theoretical studies suggest the evolution of an intermediate level of virulence. The new conclusion results from the recognition that there is often a trade-off between pathogen transmission (births) and virulence (pathogen survival) (Anderson and May 1982; May and Anderson 1983; Antia et al. 1994; Frank 1996; Koella and Restif 2001).

One indicator of reproductive success of a symbiont is the basic reproduction number (R_0), which is the mean number of new infections produced by a single infection (Anderson and May 1992; Diekmann et al. 1990; Lotz et al. 2003). It is calculated as the mean life span of an infection times the number of transmissions (equivalent to births) that would occur over that infectious period (Mollison 1995). It is very much the population growth rate of an infection and is analogous to the net reproductive rate (R_0) of free-living organisms. R_0 for both free-living and symbiotic organisms indicates population growth and therefore reproductive success (fitness). And as such, it should be maximized by natural selection.

For simplicity, we can represent transmission as β and life span as the reciprocal of the virulence α or pathogen-induced mortality. In this case, $R_0 = \frac{\beta}{\alpha}$ and R_0

increases as the infected hosts live longer (α decreases), and the infection is highly transmissible (β increases). However, there often occurs a trade-off between transmission and virulence. For example, in the case of TSV, the greater the load or intensity of the virus in a shrimp host, the greater the transmission (β increases). However, the greater the viral intensity, the greater the chance of mortality of the host (α increases) (Lotz 2010). Therefore, the trade-off is mediated by viral intensity. As a result, a balance exists between infectivity and virulence, and the maximum R_0 is obtained at intermediate levels of virulence (Fig. 2.13). This much of the theory assumes that the source of new infections is living infected hosts and that the death of the host ends infectivity (Ewald 1994; Hochberg 1998). What if transmission occurs not so much from living infected hosts but from hosts after they die? This is likely to be the case for several shrimp pathogens. Soto and Lotz (2001) and Lotz et al. (2003) demonstrated for WSSV and for TSV that transmission from dead infected shrimp is considerably greater than from living infected shrimp. This indicates that the transmissibility of a shrimp pathogen does not end with the life of the shrimp.

If transmission occurs from dead infected hosts, then R_0 is determined by the time to death of an infected host and the length of time that a dead infected host remains infectious. The infectious time of a dead shrimp depends on two factors, the ingestion of dead shrimp by other shrimp and the decay of the carcass (Soto and Lotz 2001; Lotz et al. 2003). The rate at which dead shrimp infectivity declines, whether by cannibalism or by carcass decay, is unrelated to the viral load; however, transmission rate (β) is. In this case, the load does not affect the time of infectivity of a dead infected shrimp, and the trade-off between load and time of infectivity disappears. In fact, increased virulence in live infected hosts causes a high pathogen load in dead animals and therefore increased infectivity of dead hosts without

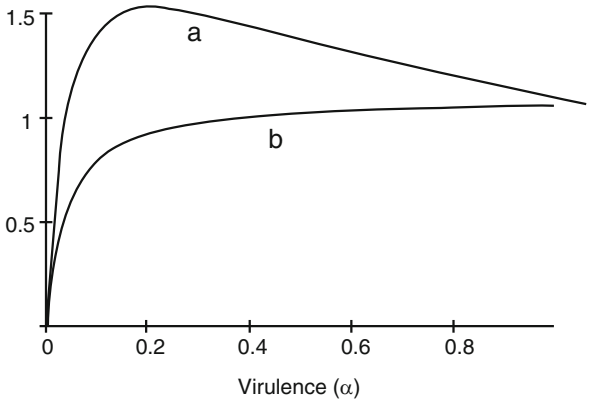

Fig. 2.13 Graph of relationship between two sources of infection (or two habitats) or two hosts assessed as pathogen-induced mortality. Curve *a* is the relationship between R_0 virulence when the source is a living host; *b* is the relationship between R_0 and virulence when the source is a dead host. Which curve obtained depends on the relative contribution of both living and dead hosts to the overall R_0

affecting the time of infectivity. So as virulence increases, R_0 also increases, and we expect virulence to increase over time if transmission is from dead shrimp (Fig. 2.13). Lotz (2010) did not consider the outcome if both living and dead hosts contribute; however, overall R_0 is $\frac{\beta_a}{\alpha} + \frac{\beta_d}{\delta}$, where β_a is the transmission rate from a living host, β_d is the transmission rate from dead hosts, α is the virulence of a pathogen to the living hosts, and δ is the infectivity decay of a dead infected host. The relative contributions of $\frac{\beta_a}{\alpha}$ and $\frac{\beta_a}{\delta}$ to R_0 are what will determine the final virulence and whether or not virulence will increase or decrease over evolutionary time. Although the above reasoning and Fig. 2.13 have been applied specifically to TSV and living and dead shrimp, they apply more generally to any two states that contribute to an overall R_0. The two states instead can be two habitats or two species of host. The state (habitat or species) that contributes the greatest to R_0 will dominate setting the optimal virulence. For species of symbiont that have both free-living and symbiotic relationships, the free-living habitat will contribute to the net reproductive rate (R_0), and the symbiotic habitat will contribute to the basic reproduction number (R_0). The two R_0s contribute to the overall growth (and thereby fitness) of the symbiont. If the two states represent different host species, then the host species that is responsible for the greatest contribution to the basic reproduction number will predominate. The conclusion is that what happens in the lesser contributing state will not be as important, and therefore very high virulence could be obtained in a host with little contribution to fitness of the symbiont. The observed virulence is a coincidental by-product of the adaptation to other habitats (Brown et al. 2012; Adiba et al. 2010). In particular, for some human bacterial pathogens, it has been postulated that bacterial pathogenicity evolved from antipredator selection by free-living bacteria and their predatory protists (Brüssow 2007; Adiba et al. 2010; Brown et al. 2012; Erken et al. 2013).

We have covered two evolutionary hypotheses to explain increased virulence of a symbiont: one contributes directly to the fitness of the symbiont, and the other is a coincidental outcome of selection for a trait important in another habitat.

Acknowledgments We thank Janet Wright, Jean Jovonovich, and Kim Overstreet for assistance. Some funding was provided by a grant from BP Exploration & Production, Inc.

References

Aanen DK, Eggleton P, Rouland-Lefèvre C, Guldberg-Frøslev T, Rosendahl S, Boomsma JJ (2002) The evolution of fungus-growing termites and their mutualistic fungal symbionts. Proc Natl Acad Sci USA 99(23):14887–14892

Adamo SA (2012) The strings of the puppet master: how parasites change host behaviour. In: Hughes D, Thomas F (eds) Parasitic manipulation. Oxford University Press, Oxford, UK, pp 36–51

Adamo SA, Webster JP (2013) Neural parasitology: how parasites manipulate host behaviour. J Exp Biol 216(1):1–2

Adiba S, Nizak C, van Baalen M, Denamur E, Depaulis F (2010) From grazing resistance to pathogenesis: the coincidental evolution of virulence factors. PLoS One 5(8):e11882

Alcivar-Warren A, Overstreet RM, Dhar AK, Astrofsky K, Carr WH, Sweeney J, Lotz JM (1997) Genetic susceptibility of cultured shrimp (*Penaeus vannamei*) to infectious hypodermal and hematopoietic necrosis virus and *Baculovirus penaei*: possible relationship with growth status and metabolic gene expression. J Invertebr Pathol 70(3):190–197

Anderson RM, May RM (1982) Coevolution of hosts and parasites. Parasitology 85(2):411–426

Anderson RM, May RM (1992) Infectious diseases of humans: dynamics and control. Oxford University Press, Oxford, UK

Antia R, Levin BR, May RM (1994) Within-host population dynamics and the evolution and maintenance of microparasite virulence. Am Nat 144(3):457–472

Antia R, Regoes RR, Koella JC, Bergstrom CT (2003) The role of evolution in the emergence of infectious diseases. Nature 426(6967):658–661

Baker AC, Schroeder DC (2008) The use of RNA-dependent RNA polymerase for the taxonomic assignment of Picorna-like viruses (Order Picornavirales) infecting *Apis mellifera* L. populations. Virol J 5:10. doi:10.1186/1743-422X-5-10

Barnard CJ, Behnke JM (eds) (1990) Parasitism and host behaviour. Taylor & Francis, London

Barrows EM (2011) Animal behavior desk reference, 3rd edn. CRC Press, Boca Raton, FL

Bates AE, Stickle WB, Harley CDG (2010) Impact of temperature on an emerging parasitic association between a sperm-feeding scuticociliate and Northeast Pacific sea stars. J Exper Mar Biol Ecol 384(1–2):44–50

Beaver PC, Jung RC, Cupp EW (1984) Clinical parasitology, 9th edn. Lea & Febiger, Philadelphia, PA

Bignell DE, Oskarsson H, Anderson JM (1980) Distribution and abundance of bacteria in the gut of a soil-feeding termite *Procubitermes aburiensis* (Termitidae, Termitinae). J Gen Microbiol 117(2):393–403

Bousfield EL, Heard RW (1986) Systematics, distribution ecology, and some host-parasite relationships of *Uhlorchestia uhleri* (Shoemaker) and *U. spartinophila*, new species (Crustacea: Amphipoda), endemic to salt marshes of the Atlantic coast of North America. J Crustacean Biol 6(2):264–274

Brown SP, Cornforth DM, Mideo N (2012) Evolution of virulence in opportunistic pathogens: generalism, plasticity, and control. Trends Microbiol 20(7):336–342

Brüssow H (2007) Bacteria between protists and phages: from antipredation strategies to the evolution of pathogenicity. Mol Microbiol 65(3):583–589

Bush AO, Heard RW Jr, Overstreet RM (1993) Intermediate hosts as source communities. Can J Zool 71(7):1358–1363

Bush AO, Lafferty KD, Lotz JM, Shostak AW (1997) Parasitology meets ecology on its own terms: Margolis et al. revisited. J Parasitol 83(4):575–583

Chouvenc T, Efstathion CA, Elliott ML, Su N-Y (2013) Extended disease resistance emerging from the faecal nest of a subterranean termite. Proc R Soc B 280(1770):20131885. doi:10.1098/rsbp.2013.1885

Cleveland LR (1926) Symbiosis among animals with special reference to termites and their intestinal flagellates. Q Rev Biol 1(1):51–60

Couch JA (1976) Attempts to increase *Baculovirus* prevalence in shrimp by chemical exposure. Prog Exp Tumor Res 20:304–314

de Bary HA (1879) Die Erscheinung der Symbiose. Karl J Tübner, Strassburg

Deardorff TL, Overstreet RM (1980a) Taxonomy and biology of species of *Goezia* (Nematoda: Anisakidae) from fishes, including three new species. Proc Helminthol Soc Wash 47(2): 192–217

Deardorff TL, Overstreet RM (1980b) *Contracaecum multipapillatum* (=*C. robustum*) from fishes and birds in the northern Gulf of Mexico. J Parasitol 66(5):853–856

Deardorff TL, Overstreet RM, Okihiro M, Tam R (1986) Piscine adult nematode invading an open lesion in a human hand. Am J Trop Med Hyg 35(4):827–830

Diekmann O, Heesterbeek JAP, Metz JAJ (1990) On the definition and the computation of the basic reproduction ratio R_0 in models for infectious diseases in heterogeneous populations. J Math Biol 28(4):365–382

Domingo E (2010) Mechanisms of viral emergence. Vet Res 41(6):38–52

Dyková I, Lom J (2007) Histopathology of protistan and myxozoan infections in fishes: an atlas. Academia, Praha

Erken M, Lutz C, McDougald D (2013) The rise of pathogens: predation as a factor driving the evolution of human pathogens in the environment. Microb Ecol 65(4):860–868

Ewald PW (1994) Evolution of infectious disease. Oxford University Press, Oxford, UK

FAO (2009a) Global aquaculture production (1950–2009). http://www.fao.org/fishery/statistics/global-aquaculture-production/query/en. Accessed 7 Aug 2014

FAO (2009b) Global capture production (1950–2009). http://www.fao.org/fishery/statistics/global-capture-production/query/en. Accessed 7 Aug 2014

Fournie JW, Overstreet RM (1993) Host specificity of *Calyptospora funduli* (Apicomplexa: Calyptosporidae) in atheriniform fishes. J Parasitol 79(5):720–727

Frank SA (1996) Models of parasite virulence. Q Rev Biol 71(1):37–78

Gaines JL Jr, Rogers WA (1972) Fish mortalities associated with *Goezia* sp. (Nematoda: Ascaridoidae) in central Florida. In: Proceedings of 25th annual conference of the Southeastern Association of Game Fish Commissioners, pp 496–497

Guo ZX, He JG, Xu HD, Weng SP (2013) Pathogenicity and complete genome sequence analysis of the mud crab dicistrovirus-1. Virus Res 171(1):8–14

Hammer HS, Stuck KC, Overstreet RM (1998) Infectivity and pathogenicity of *Baculovirus penaei* (BP) in cultured larval and postlarval Pacific white shrimp, *Penaeus vannamei*, related to the stage of viral development. J Invertebr Pathol 72(1):38–43

Hawkins WE, Solangi MA, Overstreet RM (1981) Ultrastructural effects of the coccidium, *Eimeria funduli* Duszynski, Solangi and Overstreet, 1979 on the liver of killifishes. J Fish Dis 4(4):281–295

Hochberg ME (1998) Establishing genetic correlations involving parasite virulence. Evolution 52(6):1865–1868

Hoffman GL, Landolt M, Camper JE, Coats DW, Stookey JL, Burek JD (1975) Disease of freshwater fishes caused by *Tetrahymena corlissi* Thompson, 1955, and a key for identification of holotrich ciliates of freshwater fishes. J Parasitol 61(2):217–223

Hose JE, Lightner DV, Redman RM, Danald DA (1984) Observations on the pathogenesis of the imperfect fungus, *Fusarium solani*, in the California brown shrimp, *Penaeus californiensis*. J Invertebr Pathol 44(3):292–303

Jehle JA, Abd-Alla AM, Wang Y (2013) Phylogeny and evolution of Hytrosaviridae. J Invertebr Pathol 112(Suppl 1):S62–S67

Johnson DS, Fleeger JW, Deegan LA (2009) Large-scale manipulations reveal that top-down and bottom-up controls interact to alter habitat utilization by saltmarsh fauna. Mar Ecol Prog Ser 377:33–41

Kelly DC (1982) *Baculovirus* replication. J Gen Virol 63(1):1–13

Ko RC (1976) Experimental infection of mammals with larval *Echinocephalus sinensis* (Nematoda: Gnathostomatidae) from oysters (*Crassostrea gigas*). Can J Zool 54(4):597–609

Ko RC (1977) Effects of temperature acclimation on infection of *Echinocephalus sinensis* (Nematoda: Gnathostomatidae) from oysters to kittens. Can J Zool 55(7):1129–1132

Koella JC, Restif O (2001) Coevolution of parasite virulence and host life history. Ecol Lett 4(3): 207–214

Krol RM, Hawkins WE, Overstreet RM (1990) Reo-like virus in white shrimp *Penaeus vannamei* (Crustacea: Decapoda): co-occurrence with *Baculovirus penaei* in experimental infections. Dis Aquat Organ 8(1):45–49

Labrie L, Komar C, Terhune J, Camus A, Wise D (2004) Effect of sublethal exposure to the trematode *Bolbophorus* spp. on the severity of enteric septicemia of catfish in channel catfish fingerlings. J Aquat Anim Health 14(4):231–237

LeBlanc BD, Overstreet RM (1990) Prevalence of *Baculovirus penaei* in experimentally infected white shrimp (*Penaeus vannamei*) relative to age. Aquaculture 87(3):237–242
LeBlanc BD, Overstreet RM (1991a) Effect of desiccation, pH, heat, and ultraviolet irradiation on viability of *Baculovirus penaei*. J Invertebr Pathol 57(2):277–286
LeBlanc BD, Overstreet RM (1991b) Efficacy of calcium hypochlorite as a disinfectant against the shrimp virus *Baculovirus penaei*. J Aquat Anim Health 3(2):141–145
Lefèvre T, Adamo SA, Biron DG, Missé D, Hughes D, Thomas F (2009) Invasion of the body snatchers: the diversity and evolution of manipulative strategies in host-parasite interactions. Adv Parasitol 68:45–83
Lewis EE, Campbell JF, Sukhdeo MVK (2002) The behavioural ecology of parasites. CABI Publishing, Wallingford, UK
Li L, Fröhlich J, Pfeiffer P, König H (2003) Termite gut symbiotic Archaezoa are becoming living metabolic fossils. Eukaryot Cell 2(5):1091–1098
Lightner DV (1996) A Handbook of shrimp pathology and diagnostic procedures for diseases of cultured penaeid shrimp. World Aquaculture Society, Baton Rouge, LA
Lightner DV, Redman RM (1998) Shrimp diseases and current diagnostic methods. Aquaculture 164(1):201–220
Lotz JM (2010) Evolutionary principles applied to disease control and health management in shrimp aquaculture. In: Alday-Sanz V (ed) The shrimp book. Nottingham University Press, Nottingham, UK, pp 679–694
Lotz JM, Flowers AM, Breland V (2003) A model of *Taura syndrome virus* (TSV) epidemics in *Litopenaeus vannamei*. J Invertebr Pathol 83(2):168–176
Maggenti MA, Maggenti AR, Gardner SL (2005) Online dictionary of invertebrate zoology: complete work, DigitalCommons @ University of Nebraska—Lincoln. http://digitalcommons.unl.edu/onlinedictinvertzoology/2. Accessed 7 Aug 2014
May RM, Anderson RM (1983) Epidemiology and genetics in the coevolution of parasites and hosts. Proc R Soc Lond Ser B 219(1216):281–313
McGaw IJ (2005) The decapod crustacean circulatory system: a case that is neither open nor closed. Microsc Microanal 11(1):18–36
Middlebrooks BL, Ellender RD, Lee YM (1989) Monthly assessment of lectin levels in *Penaeus aztecus* from the area of Biloxi Bay in Mississippi. J World Aquacult Soc 20(1):57A
Miller TL, Small HJ, Peemoeller B-J, Gibbs DA, Shields JD (2013) Experimental infections of *Orchitophyra stellarum* (Scuticociliata) in American blue crabs (*Callinectes sapidus*) and fiddler crabs (*Uca minax*). J Invertebr Path 114(3):346–355
Mollison D (1995) The structure of epidemic models. In: Mollison D (ed) Epidemic models: their structure and relation to data. Cambridge University Press, New York, pp 17–33
Moore J (2002) Parasites and the behavior of animals. Oxford series in ecology and evolution. Oxford University Press, New York
Noble ER, Noble GA, Schad GA, MacInnes AJ (1989) Parasitology: the biology of animal parasites, 6th edn. Lea & Febiger, Philadelphia, PA
Olson RE (1981) Effects of low temperature on the development of the microsporidan *Glugea stephani* in English sole (*Parophrys vetulus*). J Wildl Dis 17(4):559–562
Overstreet RM (1968) Parasites of the inshore lizardfish, *Synodus foetens*, from South Florida, including a description of a new genus of Cestoda. Bull Mar Sci 18(2):444–470
Overstreet RM (1982) Abiotic factors affecting marine parasitism. In: Mettrick DF, Desser SS (eds) Parasites, their world and ours: proceedings of the Fifth International Congress of Parasitology. Elsevier Biomedical Press, Amsterdam, pp 36–39
Overstreet RM (1983) Metazoan symbionts of crustaceans, Chapter 4. In: Provenzano AJ (ed) The biology of Crustacea: pathobiology, vol 6. Academic Press, New York, pp 155–250
Overstreet RM (1994) BP (*Baculovirus penaei*) in penaeid shrimps. USMSFP 10th Anniversary Review GCRL Special Publication 1:97–106
Overstreet RM (1997) Parasitological data as monitors of environmental health. Parassitologia 39(3):169–175

Overstreet RM (2013) Waterborne parasitic diseases in ocean. In: Kanki P, Grimes DJ (eds) Infectious diseases: selected entries from the Encyclopedia of sustainability science and technology. Springer Science + Business Media, New York, pp 431–496. doi:10.1007/978-1-4614-5719-0_15

Overstreet RM, Curran SS (2004) Defeating diplostomoid dangers in USA catfish aquaculture. Folia Parasitol 51(2/3):153–165

Overstreet RM, Safford S (1980) Diatoms in the gills of the commercial white shrimp. Gulf Res Rep 6(4):421–422

Overstreet RM, Stuck KC, Krol RA, Hawkins WE (1988) Experimental infections with *Baculovirus penaei* in the white shrimp, *Penaeus vannamei* (Crustacea: Decapoda), as a bioassay. J World Aquacult Soc 19(4):175–187

Overstreet RM, Hawkins WE, Deardorff TL (1995) The western mosquitofish as an environmental sentinel: parasites and histological lesions. In: Servos MR, Munkittrick KR, Carey JH, Van Der Kraak GJ (eds) Environmental fate and effects of pulp and paper mill effluents. St. Lucie Press, Delray Beach, FL, pp 495–509

Overstreet RM, Lightner DV, Hasson KW, McIlwain S, Lotz JM (1997) Susceptibility to Taura syndrome virus of some penaeid shrimp species native to the Gulf of Mexico and southeastern United States. J Invertebr Pathol 69(2):165–176

Overstreet RM, Curran SS, Pote LM, King DT, Blend CK, Grater WD (2002) *Bolbophorus damnificus* n. sp. (Digenea: Bolbophoridae) from the channel catfish *Ictalurus punctatus* and American white pelican *Pelecanus erythrorhynchos* in the USA based on life-cycle and molecular data. Syst Parasitol 52(1):81–96

Overstreet RM, Jovonovich J, Ma H (2009) Parasitic crustaceans as vectors of viruses, with an emphasis on three penaeid viruses. Integr Comp Biol 49(2):127–141

Parker JD, Spall RD, Warner MC (1971) Two new Myxosporida, *Henneguya gambusi* sp. n. and *Myxosoma pharyngeus* sp. n., in the mosquitofish, *Gambusia affinis* (Baird and Girard). J Parasitol 57(6):1297–1301

Parrish CR, Holmes EC, Moren DM, Park EC, Burke DS, Calisher CH, Laughlin CA, Saif LJ, Daszak P (2008) Cross-species viral transmission and the emergence of new epidemic diseases. Microbiol Mol Biol Rev 72(3):457–470

Pearce-Duvet JMC (2006) The origin of human pathogens: evaluating the role of agriculture and domestic animals in the evolution of human disease. Biol Rev 81(3):369–382

Pepin J (2011) The origin of AIDS. Cambridge University Press, Cambridge

Roberts LS, Janovy J Jr (2009) Gerald D Schmidt & Larry S Roberts' foundations of parasitology, 8th edn. McGraw-Hill, Boston, MA

Roekring S, Nielsen L, Owens L, Pattanakitsakul SN, Malasit P, Flegel TW (2002) Comparison of penaeid shrimp and insect parvoviruses suggests that viral transfers may occur between two distantly related arthropod groups. Virus Res 87(1):79–87

Sharp PM, Hahn BH (2010) The evolution of HIV-1 and the origin of AIDS. Philos Trans R Soc Lond B Biol Sci 365(1552):2487–2494

Shields JD, Overstreet RM (2007) Diseases, parasites, and other symbionts. Chapter 8. In: Kennedy VS, Cronin LE (eds) The blue crab, Callinectes sapidus. Maryland Sea Grant, College Park, MD, pp 299–417

Shoemaker CA, Martins ML, Xu D-H, Klesius PH (2012) Effect of *Ichthyophthirius multifiliis* parasitism on the survival, hematology and bacterial load in channel catfish previously exposed to *Edwardsiella ictaluri*. Parasitol Res 111(5):2223–2228

Small HJ, Miller TL, Coffey AH, Delaney KL, Schott E, Shields JD (2013) Discovery of an opportunistic starfish pathogen, *Ochitophrya stellarum*, in captive blue crabs, *Callinectes sapidus*. J Invertebr Pathol 114(2):178–185

Smith SJ (1981) The trematode fauna of a brackish coastal lagoon in Tasmania. PhD Dissertation, University of Tasmania, Hobart

Solangi MA, Lightner DV (1976) Cellular inflammatory response of *Penaeus aztecus* and *P. setiferus* to the pathogenic fungus, *Fusarium* sp., isolated from the California brown shrimp, *P. californiensis*. J Invertebr Pathol 27(1):77–86

Solangi MA, Overstreet RM (1980) Biology and pathogenesis of the coccidium *Eimeria funduli* infecting killifishes. J Parasitol 66(3):513–526

Solangi MA, Overstreet RM, Fournie JW (1982) Effect of low temperature on development of the coccidium *Eimeria funduli* in the Gulf killifish. Parasitology 84(1):31–39

Soto MA, Lotz JM (2001) Epidemiological parameters of *white spot syndrome virus* (WSSV) infections in *Litopenaeus vannamei* and *L. setiferus*. J Invertebr Pathol 78(1):9–15

Stickle WB, Kozloff EN, Story S (2007) Physiology of the ciliate *Orchitophrya stellarum* and its experimental infection of *Leptasterias* spp. Can J Zool 85(2):201–206

Stuck KC, Overstreet RM (1994) Effect of *Baculovirus penaei* on growth and survival of experimentally infected postlarvae of the Pacific white shrimp, *Penaeus vannamei*. J Invertebr Pathol 64(1):18–25

Stuck KC, Stuck LM, Overstreet RM, Wang SY (1996) Relationship between BP (*Baculovirus penaei*) and energy reserves in larval and postlarval Pacific white shrimp (*Penaeus vannamei*). Dis Aquat Organ 24(3):191–198

Sudhaus W (2008) Evolution of insect parasitism in rhabditid and diplogastrid nematodes. In: Makarov SE, Dimitrijević RN (eds) Advances in arachnology and developmental biology. Vienna-Belgrade-Sofia: SASA, Monographs 12, pp 143–161

Summers MD (1977) Characterization of shrimp baculovirus. EPA-600 3-77-130, Ecological research series. Environmental Research Laboratory, Gulf Breeze, FL

Thomas F, De Meeûs T, Renaud F (1996) Pattern of infection of *Gammarus aequicauda* (Amphipoda) with metacercariae of *Levinseniella tridigitata* (Trematoda: Microphallidae). J Helminthol Soc Wash 63(1):8–11

Trager W (1970) Symbiosis. Van Nostrand Reinhold Company, New York

U.S. Department of Health and Human Services, Centers for Disease Control and Prevention (2013) Intestinal parasite guidelines for domestic medical examination for newly arrived refugees. http://www.cdc.gov/immigrantrefugeehealth/guidelines/domestic/domestic-guidelines.html. Accessed 7 Aug 2014

Vidal-Martínez VM, Osorio-Sarabia D, Overstreet RM (1994) Experimental infection of *Contracaecum multipapillatum* (Nematoda: Anisakinae) from Mexico in the domestic cat. J Parasitol 80(4):576–579

Wang Y, Jehle JA (2009) Nudiviruses and other large, double-stranded circular DNA viruses of invertebrates: new insights on an old topic. J Invertebr Pathol 101(3):187–193

Wu GA, Juna SR, Sims GE, Kima SH (2009) Whole-proteome phylogeny of large dsDNA virus families by an alignment-free method. Proc Natl Acad Sci USA 106(31):12826–12831